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Involvement of the κ-opioid receptor in the anxiogenic-like effect of CP 55,940 in male rats

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Abstract

We have studied the possible interaction between three selective opioid-receptor antagonists, nor-binaltorphimine (NB: κ) (5 mg/kg), cyprodime (CY: μ) (10 mg/kg) and naltrindole (NTI: δ) (1 mg/kg), and the cannabinoid receptor agonist CP 55,940, in the modulation of anxiety (plus-maze) and adrenocortical activity (serum corticosterone levels by radioimmunoassay) in male rats. The holeboard was used to evaluate motor activity and directed exploration. CP 55,940 (75 μg/kg, but not 10 μg/kg) induced an anxiogenic-like effect, which was antagonised by NB. The other effects of CP 55,940 (75 μg/kg), a decreased holeboard activity and stimulation of adrenocortical activity, were not antagonised by any of the three opioid receptor antagonists. CY and NTI, when administered alone, induced marked reductions in motor activity, anxiogenic-like effects and stimulation of adrenocortical activity. The selective κ-opioid receptor antagonist NB, on its own, did not modify the level of anxiety but stimulated adrenocortical activity. We provide the first pharmacological evidence about the involvement of the κ-opioid receptor in the anxiogenic-like effect of CP 55,940.

Keywords: Adult male rat; CP 55,940; Selective κ-, μ- and δ-opioid antagonists; Anxiety; Adrenocortical activity

1. Introduction

Cannabinoid receptor agonists, including CP 55,940, appear to induce independent effects on motor activity and other responses related to the level of emotionality/anxiety in rodents (Onaivi et al., 1990; Arévalo et al., 2001; Romero et al., 2002). The reduction of motor activity produced by high doses of cannabinoid receptor agonists is mediated by the CB1 subtype of cannabinoid receptor, whereas the reduction of directed exploration and the anxiogenic-like effect is not reversed by the selective CB1 antagonist SR 141716A (Arévalo et al., 2001; Romero et al., 2002). The mechanism by which cannabinoid receptor agonists modulate anxiety-related behaviour remains controversial (Chaperon and Thiébot, 1999). There is evidence indicating the existence of functional interactions between the cannabinoid and the opioid systems in the modulation of analgesic responses (Manzanares et al., 1999b; Welch and Eads, 1999; Valverde et al., 2000), in addiction-related processes (Manzanares et al., 1999b; Valverde et al., 2001; Zimmer et al., 2001; Ghozland et al., 2002) and in the induction of antidepressant-like effects (Valverde et al., 2001). Cannabinoid receptor agonists enhance the release of endogenous opioids, which might account for these functional interactions (Pugh et al., 1997; Manzanares et al., 1999b; Houser et al., 2000; Valverde et al., 2001). It has been recently found that μ- and δ-opioid receptors are involved in the anxiolytic-like effect of Δ^9 tetrahydrocannabinol (THC) (Berrendero and Maldonado, 2002). Data obtained from various anxiety tests as well as from place preference/aversion paradigms suggest that the activation of the κ-receptor system induces anxiogenic/proaversive effects (De Rosset and Holtzman, 1985; Nobre et al., 2000; Sante et al., 2000). Since CP 55,940 enhances the release of dynorphins (endogenous opioid ligands acting at κ-receptors) (Pugh et al., 1997; Houser et al., 2000), we expected that the anxiogenic-like effect of CP 55,940 could be mediated by the κ-opioid receptor.

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There is substantial evidence indicating that cannabinoid receptor agonists induce an activation of the hypothalamus–pituitary–adrenal (HPA) axis in rodents (Wenger et al., 1997; Martin-Calderón et al., 1998; Manzanares et al., 1999a). It has been found that both the CB1 cannabinoid antagonist SR141716A and the nonselective opioid receptor antagonist naloxone attenuated the increases of adrenocorticotropin hormone (ACTH) and corticosterone induced by THC (Manzanares et al., 1999a). However, to our knowledge, there are no data available about the implication of different opioid-receptor subtypes in this effect. The corticotropin-releasing factor (CRF) systems appear to play a role in the mediation of the anxiogenic-like effect of cannabinoid receptor agonists (Rodriguez de Fonseca et al., 1996) and the opioid system participates in the regulation of the HPA axis (Pfeiffer and Herz, 1984). On the basis of these data, we expected that the same opioid receptor subtype involved in the anxiogenic-like effect of CP 55,940 would be also implicated in its effects on adrenocortical activity.

In the present study, we have investigated the possible implication of the three main opioid receptor subtypes (μ-, δ- and κ-receptors) in the effects of CP 55,940 on anxiety-related responses and adrenocortical activity. We used the elevated plus-maze test, which has been validated for the evaluation of anxiety in rodents, and also the holeboard, which provides additional information about motor activity and site-directed exploration (Pellow et al., 1985; File, 1992).

2. Material and methods

2.1. Animals, experimental conditions and pharmacological treatments

Experiments were performed on Wistar albino adult male rats of approximately 100 days of age, which were served by Harlan Interfauna Ibérica (Barcelona, Spain). The animals were maintained at a constant temperature of 20 °C and in a reverse 12-h dark/light cycle (lights on at 18:30 h), with free access to food (commercial diet for rodents A04; Panlab, Barcelona, Spain) and water. Animals were housed in standard laboratory cages, each one containing groups of five to six individuals, and were habituated to the environmental conditions during a 15-day period. On the day of testing, the animals were habituated in a quiet laboratory for a 30-min period before experimental procedures began. Behavioural tests were carried out under the same illumination conditions as those in the animal facilities (red light).

In a first experiment, we studied the behavioural effects of CP 55,940 (Tocris) and its possible interaction with the κ-opioid receptor antagonist nor-binaltorphimine (NB) (Sigma Aldrich). Two doses of CP 55,940 were used, 75 and 10 μg/kg. The higher dose of CP 55,940 (75 μg/kg) was chosen on the basis of previous data indicating that, at this dose, the compound induces anxiogenic-like effects in rats (Arévalo et al., 2001). Since other cannabinoid receptor agonists have been shown to be anxiolytic at low doses (Onaivi et al., 1990; Rodriguez de Fonseca et al., 1996; Berrendero and Maldonado, 2002), we also used a lower dose of CP 55,940 (10 μg/kg) to study a possible biphasic effect on plus-maze activity. The cannabinoid receptor agonist CP 55,940 was administered intraperitoneally 30 min before behavioural testing (holeboard immediately followed by the plus-maze). The κ-opioid receptor antagonist NB (5 mg/kg) was administered intraperitoneally 3 h before the administration of CP 55,940. In a second experiment, we performed an additional series of interaction studies to address the possibility that any of the other main opioid receptor subtypes (μ and δ) could be involved in the anxiogenic-like effect of the higher dose of CP 55,940. The μ-opioid receptor antagonist cyprodime (CY) (10 mg/kg) (provided by Dr. Schmidhammer) and the δ-opioid receptor antagonist naltrindole (NTI) (1 mg/kg) (Sigma Aldrich) were injected subcutaneously immediately before CP 55,940. As in the first experiment, behavioural testing was performed 30 min after the administration of the cannabinoid receptor agonist. The doses of the three opioid receptor antagonists and the corresponding pretreatment times were chosen on the basis of previous studies where the antagonists have been shown to antagonise effectively opiate effects (Sofuoglu et al., 1991; Endoh et al., 1992; Schmidhammer, 1998). The cannabinoid receptor agonist CP 55,940 was dissolved in ethanol:cremophor:-saline (1:1:18) (cremophor, Fluka BioChemiKa), whereas CY, NTI and NB were dissolved in 0.9% saline solution. In all cases, the volume administered was 1 ml/kg and appropriate control groups were used, which received the same number of injections with the corresponding vehicles. We measured the serum corticosterone levels of the animals receiving the higher dose of CP 55,940 (75 μg/kg) and of the groups employed for the corresponding μ-, δ- and κ-interaction studies. We assigned (at random) 10 animals per group for the endocrine determinations. All experimental procedures were carried out between 09:00 and 14:30 h.

The experiments performed in this study are in compliance with the Royal Decree 223/1988 of 14 March (BOE 18) and the Ministerial Order of 13 October 1989 (BOE 18) about protection of experimental animals, as well as with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Holeboard

The holeboard was a box (60 × 60 × 45 cm) with matte-painted metallic walls and a plastic-covered wooden floor bearing four equally spaced holes (3.8 cm in diameter) and divided into 36 squares (10 × 10 cm). The duration of the test was of 5 min. The parameters recorded were external ambulation (number of line crossings in the periphery, by the walls, horizontal activity), frequency of rearings (number of times that the animal stood on its rear limbs, vertical

activity), and frequency and duration (s) of head-dipping. This test provides independent measures of motor activity (external ambulation and rearing) and directed exploration (head-dipping frequency and duration) (File, 1992).

2.3. Plus-maze

The plus-maze consisted of two open arms (50 × 10 cm) and two enclosed arms of the same size with 40-cm high walls arranged so that the arms of the same type were opposite each other. The junction of the four arms formed a central square area (10 × 10 cm). The apparatus was made of hard plastic material and elevated to a height of 62 cm. The test was carried out for 5 min. The measures recorded were frequency and duration of arm visits, separately for open and closed arms. An arm was considered to be visited when the animal entered it with the four limbs. We also estimated the percentage of entries into the open arms to the total number of entries and the percentage of time spent in the open arms to the total time in arms. In this test, the percentage of time in the open arms of the maze provides the best measure of anxiety, whereas the number of closed arms entries provides the best measure of motor activity (Pellow et al., 1985; File, 1992).

Each test was started by placing the animals in the area of the apparatus considered more behaviourally neutral [one of the corners (holeboard), at the centre of the apparatus facing one of the enclosed arms (plus-maze)] so that the animal was not artificially induced to perform a significant pattern. The two apparatus were thoroughly cleaned at the end of every test.

2.4. Corticosterone assay

Three minutes after completion of the plus-maze, the animals were killed by decapitation. Blood samples were collected from the trunk and centrifuged (3000 rpm for 15 min), and serum was stored at − 80 °C. Corticosterone was measured using a solid phase ^{125}I radioimmunoassay (Coat-A Count Rat Corticosterone kit, Diagnostic Products, Los Angeles, CA). The detection limit was 5.7 ng/ml and the intraassay and interassay coefficients of variation were less than 10%.

2.5. Statistical analysis

In order to address possible interactions between CP 55,940 and the different opioid receptor antagonists, all data were analysed by two-way analysis of variance (ANOVA), with the two factors being opioid receptor antagonist and cannabinoid receptor agonist. The data were previously tested for normality (Kolmogorov–Smirnov test) and equal variance (Levene median test). In those cases in which the data did not fulfil these criteria, we used a rank transform before performing the ANOVA. Since the experimental protocol (pretreatment times) was the same for the interaction studies with CY and NTI, the groups injected with saline + vehicle and saline + CP 55,940 were the same for both studies. Therefore, the results obtained in these two experiments were first analysed as a whole by two-way ANOVAs. Where appropriate (see results), the effect of each antagonist (CY and NTI) was analysed separately by additional ANOVAs. Some animals (about one per group) accidentally fell from the plus-maze and were excluded from the analysis. Thus, the degrees of freedom were different for plus-maze and holeboard. The Student–Newman–Keuls (SNK) test with a level of significance set at $P < .05$ was used for post-hoc comparisons.

3. Results

When the results from the ANOVA included a significant interaction between factors (Antagonist × Agonist), the SNK test provided comparisons between the mean values appearing in tables and figures (mean values corresponding to the different experimental groups). In these cases, significant differences are represented by standard statistical symbols. When the ANOVA showed significant overall effects of the agonist and/or the antagonist but the interaction between factors was not significant, the SNK test compared overall mean values that do not appear in the tables/figures. In these cases, the significant effects are indicated in the footnotes of tables and figures and explained in detail in the text.

3.1. First experiment: interaction study with NB

The analysis of the results obtained in the holeboard showed significant overall effects of CP 55,940 on external ambulation [$F(2,75) = 8.4$, $P < .001$], rearing frequency [$F(2,75) = 20.4$, $P < .001$], head-dipping frequency [$F(2,75) = 7.5$, $P = .001$] and head-dipping duration [$F(2,75) = 3.6$, $P < .05$]. No significant effect of NB or interaction between NB and CP 55,940 were found for any of the parameters measured in this test. Post-hoc comparisons indicated that, whether or not the rats were given NB, the two doses of CP 55,940 significantly reduced external ambulation and head-dipping frequency, whereas rearing frequency and head-dipping duration were significantly reduced by only the 75 μg/kg dose. A significant difference between the two doses of the cannabinoid agonist was found for rearing frequency (Table 1).

With respect to the plus-maze, the two-way ANOVA performed on the percentages of time in open arms indicated a significant interaction between NB and CP 55,940 [$F(2,67) = 3.6$, $P < .05$]. Post-hoc comparisons revealed that the higher dose of CP 55,940 (75 μg/kg) significantly decreased the percentage of time spent in open arms, and that the κ-opioid receptor antagonist NB antagonised this effect (Fig. 1A). No significant effects were found for either the lower dose of CP 55,940 (10 μg/kg) or for the κ-receptor

Table 1
Effects of CP 55,940 and NB on holeboard activity in male rats

	External ambulation	Rearing frequency	Head-dipping frequency	Head-dipping duration (s)
SS + Vh (15)	123.7 ± 8.0	24.4 ± 1.7	9.7 ± 1.4	21.3 ± 3.7
SS + CP_{10} (15)	97.1 ± 13.7	19.1 ± 2.5	7.1 ± 1.2	17.1 ± 2.7
SS + CP_{75} (15)	79.5 ± 12.5	7.2 ± 2.2	5.2 ± 1.0	14.5 ± 3.6
NB + Vh (10)	113.7 ± 11.7	21.2 ± 2.3	9.3 ± 1.8	18.5 ± 4.5
NB + CP_{10} (14)	80.4 ± 9.3	19.3 ± 2.0	5.4 ± 1.0	10.9 ± 2.2
NB + CP_{75} (12)	60.3 ± 12.7	9.3 ± 3.1	3.8 ± 1.3	8.5 ± 3.1

Values represent the mean ± S.E.M. from the number of animals indicated in parenthesis. CP = CP 55,940 (10 and 75 μg/kg), NB = nor-binaltorphimine (5 mg/kg). The animals received an injection with either NB or saline (SS) 3 h before the administration of either CP or vehicle (Vh) (see text). The holeboard was performed 30 min after the administration of CP.
See text for overall effects of CP and differences between doses.

Table 2
Effects of CP 55,940, CY and NTI on holeboard activity in male rats

	External ambulation	Rearing frequency	Head-dipping frequency	Head-dipping duration (s)
SS + Vh (14)	124.4 ± 10.6	22.2 ± 1.6	10.2 ± 1.0	26.1 ± 3.8
SS + CP_{75} (17)	81.3 ± 14.6	6.5 ± 1.5*	3.5 ± 1.2	7.7 ± 2.7
CY + Vh (9)	62.9 ± 16.9	10.1 ± 2.6*	6.9 ± 1.3	14.6 ± 3.7
CY + CP_{75} (11)	37.4 ± 11.3	6.4 ± 2.0*	5.6 ± 2.5	19.2 ± 11.3
NTI + Vh (11)	76.7 ± 15.2	12.9 ± 2.4*	7.1 ± 1.6	13.4 ± 3.1
NTI + CP_{75} (11)	58.4 ± 17.1	3.7 ± 0.9*,#	3.2 ± 1.1	5.1 ± 1.6

Values represent the mean ± S.E.M. from the number of animals indicated in parenthesis. CP_{75} = CP 55,940 (75 μg/kg), CY = cyprodime (10 mg/kg), NTI = naltrindole (1 mg/kg). The animals received an injection with either the antagonist (CY/NTI) or saline (SS) immediately before the administration of either CP or Vh (see text). The holeboard was performed 30 min after the administration of CP.
See text for overall effects of CP and the antagonists.
* Student–Newman–Keuls: $P < .05$ vs. SS + Vh.
# Student–Newman–Keuls: $P < .05$ vs. NTI + Vh.

antagonist. With respect to the percentages of entries in the open arms, the interaction between factors did not reach the level of statistical significance [$F(2,67) = 2.5$, $P = .09$]. However, a visual inspection of the data shows that the tendencies were similar to the results obtained in the percentage of time spent in these arms (Fig. 1A and B). The analysis of the closed-arm entries indicated a significant overall effect of NB [$F(1,67) = 4.6$, $P < .05$]. As Fig. 1C shows, the κ-opioid receptor antagonist decreased this parameter. No significant effect of CP 55,940 or interaction between NB and CP 55,940 were found.

3.2. Second experiment: interaction studies with CY and NTI

The analysis of the results obtained in the holeboard revealed significant overall effects of CP 55,940 (75 μg/kg) on external ambulation [$F(1,67) = 5.8$, $P < .05$], rearing frequency [$F(1,67) = 39.4$, $P < .001$], head-dipping frequency [$F(1,67) = 10.4$, $P < .01$] and head-dipping duration [$F(1,67) = 17.4$, $P < .001$]. As Table 2 shows, the cannabinoid receptor agonist reduced all the parameters measured in this test. The ANOVA also revealed significant overall effects of the factor antagonist on external ambulation [$F(2,67) = 7.2$, $P = .001$] and rearing frequency [$F(2,67) = 8.1$, $P < .001$]. The SNK indicated that the two opioid receptor antagonists CY and NTI significantly decreased the two parameters. The interaction between factors was only significant for rearing frequency [$F(2,67) = 5.5$, $P < .01$]. As Table 2 shows, the cannabinoid receptor agonist CP 55,940 and NTI appeared to produce additive effects on rearing behaviour.

The ANOVA performed on the percentages of time in open arms (plus-maze) indicated that the interaction between factors was on the limit of the statistical significance [$F(2,63) = 2.9$, $P = .06$], and similar results were obtained for the two main factors (agonist and antagonist). A visual inspection of the data represented in Fig. 2A shows that, as in the first experiment, CP 55,940 clearly decreased the percentage of time spent in the open arms, and that each opioid receptor antagonist (CY and NTI) appeared to

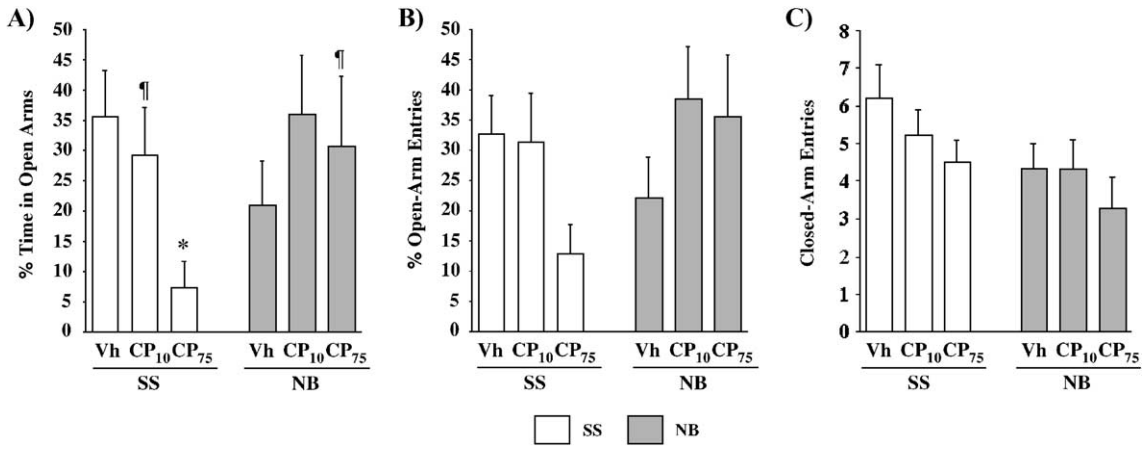

Fig. 1. Effects of CP 55,940 (10 and 75 μg/kg) and NB (5 mg/kg) on plus-maze activity in male rats. The animals received an injection with either NB or saline (SS) 3 h before the administration of either CP or vehicle (Vh) (see text). The plus-maze was performed immediately after the holeboard. Histograms represent the mean ± S.E.M. from the following number of animals: SS + Vh (14), SS + CP_{10} (13), SS + CP_{75} (14), NB + Vh (10), NB + CP_{10} (12), NB + CP_{75} (10). Student–Newman–Keuls: * $P < .05$ vs. SS + Vh, ¶ $P < .05$ vs. SS + CP_{75}. See text for the overall effect of NB on closed-arm entries.

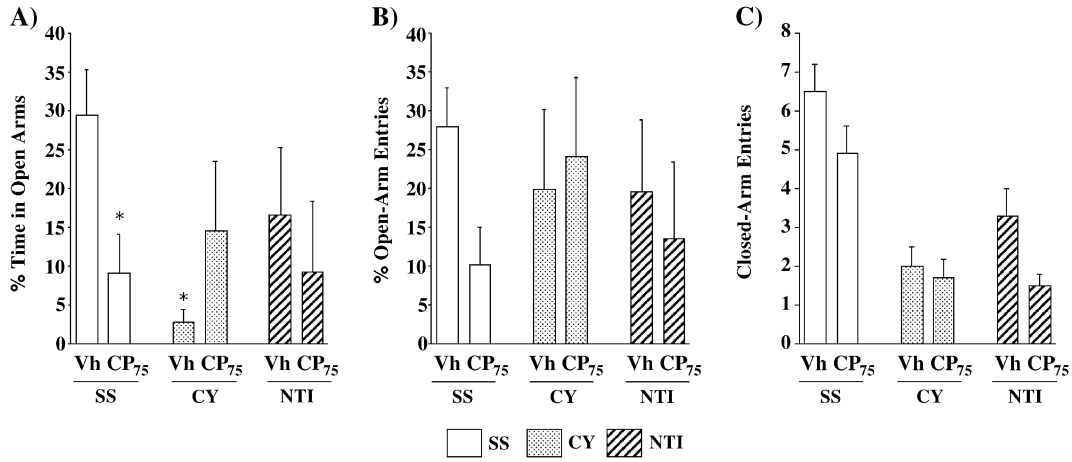

Fig. 2. Effects of CP 55,940 (75 μg/kg) (CP_{75}), CY (10 mg/kg) and NTI (1 mg/kg) on plus-maze activity in male rats. The animals received an injection with either the antagonist (CY/NTI) or saline (SS) immediately before the administration of either CP or Vh (see text). The plus-maze was performed immediately after the holeboard. Histograms represent the mean ± S.E.M. from the following number of animals: SS + Vh (14), SS + CP_{75} (14), CY + Vh (8), CY + CP_{75} (11), NTI + Vh (11), NTI + CP_{75} (11). Student–Newman–Keuls: * $P < .05$ vs. SS + Vh. See text for overall effects of CP and the antagonists.

interact in a distinct manner with the cannabinoid receptor agonist. Thus, we attributed the results of the ANOVA to a type II error. In order to clarify the effects of the three drugs, we analysed further, by separate ANOVAs, the studies with CY and NTI. As expected, we found significant overall effects of CP 55,940 [$F(1,46) = 6.7$, $P = .01$] and NTI [$F(1,46) = 4.2$, $P < .05$], as well as a significant interaction between CY and CP 55,940 [$F(1,43) = 6.4$, $P < .05$]. The three compounds (CP 55,940, CY and NTI) reduced the percentage of time in the open arms (Fig. 2A). With respect to the percentage of entries in the open arms, the ANOVA performed with the four groups corresponding to the NTI study was also more powerful than the ANOVA including both, the NTI and the CY studies, and a significant overall effect of CP 55,940 was found [$F(1,46) = 4.8$, $P < .05$]. As Fig. 2B shows, CP 55,940 reduced the percentage of entries in the open arms. The separate analysis including the groups receiving CY did not detect any significant effect on this parameter. The analysis of the closed-arm entries revealed significant overall effects of both, CP 55,940 [$F(1,63) = 7.2$, $P < .01$] and the factor antagonist [$F(2,63) = 29.7$, $P < .001$], whereas the interaction between factors was not significant. The three compounds, CP 55,940 and the two antagonists (CY and NTI), reduced the number of entries in the closed arms (Fig. 2C).

Table 3
Effects of CP 55,940, NB, CY and NTI on serum corticosterone levels of male rats

	Corticosterone levels (ng/ml)
SS + Vh (10)	520.3 ± 25.2
SS + CP_{75} (10)	723.1 ± 16.7
NB + Vh (10)	684.9 ± 25.4
NB + CP_{75} (10)	838.1 ± 35.7
SS + Vh (10)	668.2 ± 26.8
SS + CP_{75} (10)	732.1 ± 32.8
CY + Vh (8)	814.5 ± 55.2
CY + CP_{75} (10)	851.2 ± 41.2
NTI + Vh (10)	744.9 ± 42.7
NTI + CP_{75} (10)	841.8 ± 26.2

Values represent the mean ± S.E.M. from the number of animals indicated in parenthesis. SS = saline, Vh = vehicle (see text), CP_{75} = CP 55,940 (75 μg/kg), NB = nor-binaltorphimine (5 mg/kg), CY = cyprodime (10 mg/kg), NTI = naltrindole (1 mg/kg). NB was injected 3 h before the administration of CP, whereas CY and NTI were injected immediately before the cannabinoid receptor agonist. The animals were sacrificed by decapitation at the end of behavioural testing.
See text for overall effects of CP and the antagonists.

3.3. Corticosterone determinations

The analysis of the data from the NB study showed significant overall effects of both, CP 55,940 [$F(1,36) = 44.8$, $P < .001$] and NB [$F(1,36) = 27.6$, $P < .001$] on corticosterone levels. No significant interaction between NB and CP 55,940 was found. As Table 3 shows, the two compounds increased the serum corticosterone levels. The analysis of the results obtained in the studies with CY and NTI confirmed the effect of CP 55,940 [$F(1,52) = 4.6$, $P < .05$] and revealed a significant overall effect of the factor antagonist [$F(2,52) = 6.5$, $P < .01$]. No significant interaction between factors was found. The three compounds (CP 55,940, CY and NTI) induced significant increases in the corticosterone levels (Table 3).

4. Discussion

The cannabinoid receptor agonist CP 55,940 at the highest dose used in this study (75 μg/kg) induced anxiogenic-like responses in the plus-maze. This anxiogenic-like effect was indicated by a significant reduction of the percentage of time spent in the open arms (the best measure of anxiety in

this test), and a clear tendency to reduced percentages of entries in open arms. It has been proposed that the percentage of time spent in the open arms is more sensitive to drug effects than the number of entries (Pellow et al., 1985). Previous studies have shown that cannabinoid receptor agonists, including CP 55,940, exert anxiogenic-like effects in the plus-maze and other tests of anxiety (Onaivi et al., 1990; Rodriguez de Fonseca et al., 1996; Giuliani et al., 2000; Arévalo et al., 2001). In addition, cannabis has well-documented effects of producing paranoia, panic and anxiety in a proportion of human users (Hollister 1986). In rats, CP 55,940 increases Fos immunoreactivity in brain structures known to be involved in anxiety and fear-related responses such as the central nucleus of the amygdala, the periaqueductal gray and the paraventricular nucleus of the hypothalamus (Arnold et al., 2001). At the dose of 75 μg/kg, CP 55,940 also produced a decrease in holeboard activity (horizontal and vertical activity and exploration), whereas at 10 μg/kg it induced a moderate reduction of only external ambulation and head-dipping frequency. It has been shown that this cannabinoid receptor agonist produced depression of motor activity in the 25–100 μg/kg dose range (McGregor et al., 1996; Arnold et al., 1998). However, the results obtained with the lower dose (10 μg/kg) are more controversial. Previous studies have shown either a stimulatory effect (McGregor et al., 1996) or a lack of effect (Arnold et al., 1998). Thus, it seems that the effects of low doses of CP 55,940 largely depend on the specific experimental conditions. There is no report in the literature of anxiolytic-like effects of CP 55,940 in adult animals. However, low doses of other cannabinoid receptor agonists such as nabilone (Onaivi et al., 1990), HU-210 (Rodriguez de Fonseca et al., 1996) and THC (Berrendero and Maldonado, 2002) can induce anxiolytic-like effects. Under the present experimental conditions, a low dose of CP 55,940 (10 μg/kg) did not induce any significant effect on anxiety-related responses in the plus-maze. It is possible that at lower doses and/or under different experimental conditions, an anxiolytic-like effect of this compound can be achieved.

Previous results indicate that central CRF participates in some cannabinoid-induced anxiety-related responses, such as defensive withdrawal behaviour (Rodriguez de Fonseca et al., 1996). It has been also proposed that the benzodiazepine/GABA A receptor may be involved in both the anxiogenic and the anxiolytic reactions to various cannabinoid receptor agonists in the plus-maze (Onaivi et al., 1990). Recently, it has been reported that the μ-opioid receptor antagonist β-funaltrexamine (5 mg/kg) and the δ-opioid receptor antagonist NTI (2.5 mg/kg), but not the κ-opioid receptor antagonist NB (2.5 mg/kg) abolished THC anxiolytic-like effects in the light-dark box (Berrendero and Maldonado, 2002). In the present study, the anxiogenic-like effect of CP 55,940 in the plus-maze was antagonised by the κ-opioid receptor antagonist NB, indicating the mediation of κ-receptors. NB on its own did not affect the anxiety-related responses in this test. This result agrees with genetic studies indicating that the κ-opioid receptor does not play a major role in the tonic modulation of anxiety (Filliol et al., 2000). The κ-opioid system appears to be involved in other effects of cannabinoid receptor agonists. It has been shown that CP 55,940 induces the release of dynorphins (Pugh et al., 1997; Houser et al., 2000), which through activation of κ-opioid receptors participate in the antinociceptive effect of the cannabinoid receptor agonist. Recent studies in dynorphin-deficient mice (Zimmer et al., 2001) and κ-deficient mice (Ghozland et al., 2002) have revealed that the absence of either dynorphin or κ-receptors suppressed the negative motivational effects of THC in place conditioning paradigms. Moreover, NB also blocked the establishment of THC-induced conditioned place aversion (Zimmer et al., 2001). Concordant with these studies, our results indicate that an activation of the dynorphin–κ receptor system likely participates in the anxiogenic-like effect of CP 55,940. In fact, the activation of κ-receptors either by exogenous agonists or by endogenous dynorphins induces anxiety and aversive responses (De Rosset and Holtzman, 1985; Anseloni et al., 1999; Nobre et al., 2000; Sante et al., 2000). The present results also show that the effects of CP 55,940 on holeboard activity were not antagonised by NB. Thus, the κ-receptors are specifically involved in the anxiogenic-like effect of the cannabinoid receptor agonist, whereas other behavioural effects of the compound (decreased motor activity and exploration) are independent of this opioid receptor. This result further supports the view that distinct mechanisms subserve the effects of CP 55,940 on motor activity, exploration, and anxiety. Under the present experimental conditions, the behavioural effects of CP 55,940 were not antagonised by either CY or NTI indicating that they did not depend on the activation of μ- or δ-opioid receptors. We cannot exclude the possibility that lower doses of CY and NTI would have interacted differently with the cannabinoid receptor agonist.

CY and NTI, when administered alone, produced marked decreases in motor activity (in the two behavioural tests) and induced anxiogenic-like responses in the plus-maze. These results are in agreement with previous studies (Kelley et al., 1996; Leventhal et al., 1996; Fernández et al., 2000; Skoubis et al., 2001), and support the view that μ- and δ-receptors are involved in the tonic control of motor activity and anxiety/aversion-related responses. NB, on its own, decreased locomotor activity in the plus-maze but it did not affect holeboard activity, indicating that this behavioural effect of the κ-receptor antagonist depends on the specific test employed.

According to previous studies on the effects of several cannabinoid receptor agonists on adrenocortical activity (Wenger et al., 1997; Martin-Calderón et al., 1998; Manzanares et al., 1999a), the present results indicate that CP 55,940 induced a significant increase in the serum corticosterone levels. This effect was not reversed by any of the three selective opioid receptor antagonists. It has been reported that naloxone reduced the corticosterone responses induced by central administration of THC in rats (Manza-

nares et al., 1999a). These apparently contradictory results might be attributable, at least in part, to the lack of selectivity of naloxone, and/or to the different cannabinoid receptor agonist employed. The three selective opioid receptor antagonists used in the present study induced increases in the corticosterone levels when administered alone. These results agree with previous studies on the effects of various opioid receptor antagonists on adrenocortical activity (Eisenberg, 1984; Pfeiffer and Herz, 1984; Bailey and Kitchen, 1987; Cover and Buckingham, 1989). As a whole, the present results indicate that the cannabinoid receptor agonist and the three selective opioid receptor antagonists increased corticosterone levels, although with different effects on anxiety. The κ-opioid receptor antagonist NB antagonised the anxiogenic-like effect of CP 55,940, but not its effect on adrenocortical activity. It is likely that the effects of cannabinoid receptor agonists on anxiety and adrenocortical activity are mediated by distinct mechanisms.

In conclusion, the selective κ-opioid antagonist NB antagonised the anxiogenic-like effect of CP 55,940 in the plus-maze, but it did not reverse other effects of the cannabinoid agonist (a decrease in holeborad activity and stimulation of adrenocortical activity). We provide the first pharmacological evidence about the involvement of the κ-opioid receptor in the anxiogenic-like effect of a cannabinoid agonist.

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