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Acute administration of the CB_1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat

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ANIMAL models have revealed that psychoactive cannabinoids induce both anxiolytic and anxiety-like reactions which are dose- and context-dependent. In the present study we examined the acute actions of the CB_1 cannabinoid receptor antagonist SR 141716A in both the defensive withdrawal test and the elevated plus-maze in rats. Acute administration of SR 141716A (0.1, 1 and 3 mg kg^{-1}) induced defensive responses in both anxiety tests, at a dose of 3 mg kg^{-1}. This dose had no effect on horizontal locomotor activity and did not activate the hypothalamus–pituitary–adrenal axis, although several cannabinoid withdrawal-like behavioural symptoms were observed. These results demonstrate that blockade of the endogenous cannabinoid tone might induce anxiety-like responses in rats.

Key words: Anxiety; Behaviour; Cannabinoids; CB_1 receptors; Corticosterone; Defensive withdrawal; Plus-maze; SR 141716A

Introduction

Acute exposure to cannabis derivatives (hashish, marijuana) often results in subjective emotional responses.[1] Whereas most cannabis users report a placid relaxation after acute marijuana smoking, the most frequent adverse psychological consequence of cannabis consumption is the onset of an acute panic reaction.[1,2] The acute administration of psychotropic cannabinoids such as (–)-Δ^9-tetrahydrocannabinol, the main psychoactive constituent of marijuana, may also cause anxiogenic responses in humans.[3] Psychoactive cannabinoids display a dose-dependent biphasic profile in laboratory animals when studied using standard anxiety tests. Low doses of these compounds may induce anxiolysis, whereas higher doses result in anxiety-like reactions: such results have been obtained using the elevated plus-maze,[4] the defensive withdrawal test[5] or the dark–light emergence test.[6] The neurobiological substrate of cannabinoid-induced emotional responses remains to be elucidated, although they are likely to be mediated through the activation of brain cannabinoid receptors,[7] CB_1. These receptors are present in the limbic system and brain nuclei related to the stress response[8] such as central amygdala or paraventricular nucleus of the hypothalamus. Psychotropic cannabinoids are also potent activators of the hypothalamus–pituitary–adrenal (HPA) axis,[9,10] which might contribute to the unpleasant effects described after smoking marijuana. Interestingly, anandamide, a putative endogenous ligand for CB_1 receptors[11] also activates the HPA axis by inducing the release of corticotropin-releasing factor (CRF) from the hypothalamus.[12] However, no study has addressed the possible role of the endogenous anandamide-CB_1 systems in the brain in the regulation of emotional responses.

The recently described cannabinoid antagonist SR 141716A[13] has provided a tool for examining the effects of the blockade of the endogenous cannabinoid tone in naive animals. Using this compound it has been recently demonstrated that CB_1 receptors are involved in the regulation of arousal and the sleep–waking cycle.[14] They also regulate the population activity of the A_{10} mesencephalic nigrostriatal dopamine neurones controlling extrapyramidal function.[15] CB_1 receptors were also found to mediate the discriminative stimulus properties of psychoactive cannabinoids.[16] Studies performed in mice have revealed that SR 141716A is able of stimulating

locomotor activity in intact animals, suggesting the presence of an inhibitory endogenous cannabinoid tone regulating spontaneous activity.[17]

The present study was designed to explore the effect of SR 141716A-induced blockade of CB_1 receptors in the performance of male rats in two behavioural tests which are very sensitive to the anxiety-like reactions induced by cannabinoids, the defensive withdrawal test under familiar conditions,[5] and the elevated plus-maze.[4] We also explored the endocrine response by monitoring plasma levels of stress-related hormones such as adrenocorticotropin hormone (ACTH), corticosterone and prolactin. The occurrence of cannabinoid withdrawal-related behaviours[18] such as grooming, wet dog shakes, facial rubbing, scratching sequences or abdominal stretching was also monitored to characterize further the behavioural effects of SR 141716A in rats.

Methods

Animals: Male Wistar rats (Panlab, Barcelona, Spain) weighing 350 ± 35 g at the start of the experiment were housed individually and maintained in a temperature- and light-controlled environment on a 12:12 h light:dark cycle (lights on at 08:00 h) with free access to food and water. Animals were allowed at least a 2-week period for acclimatization to the animal room. They were subsequently handled daily for a week before the beginning of the experimental sessions. All the procedures were carried out according to the EEC ethical regulations for animal research.

Drugs: SR 141716A (*N*-(piperidino-5-(4-chlorophenyl)-4-methylpyrazole-3-carboxamide hydrochloride) was obtained through Sanofi Recherche, Montpellier, France. It was suspended in distilled water with two drops of Tween 80 as vehicle and made up daily to the appropriate concentrations to be administered i.p. in a volume of 5 ml kg^{-1}.

Behavioural testing: All the behavioural studies took place during the morning time of the light cycle (09:30–13:00 h).

Defensive withdrawal: Defensive withdrawal test was conducted as previously described.[5,19] The apparatus consisted of an opaque open field (100 × 100 × 40 cm), the floor of which was marked with 20 × 20 cm squares. The field contained a cylindrical polyethylene chamber 17 cm deep and 10 cm in diameter. The chamber was opened at one end and situated along the wall running lengthside and 20 cm away from a corner of the open field. The open field was illuminated using a 500 W ceiling halogen light which was regulated to yield 350 lux at the centre of the open field. Testing was conducted under both novelty and familiar conditions. The latter was achieved by habituating the animals for 10 min to the open field without the small chamber 24 h before the test session. The rats were placed inside the chamber, in the open field, and the following behaviours were scored by trained observers, who were blind to experimental conditions: the latency to leave the chamber (emergence latency), defined as placement of all four paws in the open field; the total time spent in the chamber; the mean time spent in the chamber (i.e., the total time spent in the chamber divided by the total number of entries); motor activity, defined as the total number of lines on the floor of the open field crossed outside the chamber (crossings), and the number of rearings performed outside the chamber. The test length was 10 min for the novelty condition and 15 min for the field-habituated animals. After testing each animal, the apparatus was cleaned with a weak acid solution (1% acetic acid), to prevent olfactory cues from affecting the behaviour of subsequently tested rats.

The effects of acute i.p. administration of SR 141716A (0.1, 1 and 3 mg kg^{-1}) on the defensive withdrawal test were evaluated under either novelty and familiar (field-habituated) conditions, 30 min after the injection of the cannabinoid antagonist. A separate group of animals was killed 60 min after the administration of SR 141716A, trunk blood was collected, and plasma obtained for the determination of corticosterone, ACTH and prolactin.

Elevated plus-maze: The performance of the animals in the elevated plus maze was evaluated following previously described methods.[20,21] The apparatus consisted of two wooden open arms and two enclosed arms (40 cm high) of the same size (50 × 10 cm) which were arranged so that the arms of the same type opposite each other. The maze was elevated 50 cm above the floor. The test procedure started by placing each animal in the centre of the maze, facing a closed arm. The test length was 5 min and the total time spent in each arm and the number of arm entries were scored by trained observers. The results were expressed as the percentage of time spent in the exposed arms over the total time spent in the open + closed arms of the maze, and the number of arm entries.

Locomotor activity: Locomotor activity was evaluated using photocell cages of 40 × 25 cm. Animals were habituated for 8 h to the cages 24 h before the testing session. Animals were studied for 2 h and the number of beambreaks and crossovers was automatically obtained each 10 min.

Cannabinoid withdrawal-related behaviours: The occurrence of cannabinoid withdrawal-related behaviours[18] were achieved using observational boxes of 40 × 40 × 35 cm. Animals were placed in the box after the i.p. injection of either vehicle or SR 141716A, and observed for 1 h. The following behaviours were scored by trained observers, blind to experimental conditions: time spent grooming, and the number of rearings, wet dog shakes, facial rubbings, scratching sequences and abdominal stretchings.

Hormonal determinations: Rats were killed by rapid decapitation using a guillotine. Animals were previously familiarized with this handling procedure. Trunk blood was collected in tubes containing 400 μl of 6% EDTA, and centrifuged at 2500 × *g* at 4°C. Plasma was stored frozen at –20°C until assayed. Plasma corticosterone levels were measured by a radioimmunoassay system (RIA), using a specific antibody from Bio Clin (Cardiff, England) with hydroxyapatite for separating the free and bound phases. This RIA system yields basal values of corticosterone of 175 ± 25 ng ml^{-1} in undisturbed adult male animals, and 500 ± 70 ng ml^{-1} in stressed animals.[5] The variability of the method was 15.3%, and the detection limit was 62 pg ml^{-1}. Plasma ACTH levels were measured using a commercial kit (CIS-Biointernational, Gif-Sur-Yvette, France). The intra-assay coefficient of variation was 5% and the sensitivity was 10 pg ml^{-1}. Plasma prolactin (PRL) levels were measured by a specific double-antibody RIA system using materials kindly provided by the NIH (Bethesda, MD, USA). Values are expressed in ng ml^{-1} of reference preparation rPRL-RP-3. The intra-assay coefficient of variation was 3.3% and the sensitivity was 0.025 ng ml^{-1}. All samples were measured in the same assay to avoid interassay variations. Details on the methods have been published elsewhere.[10]

Statistics: Data were assessed by analysis of variance (ANOVA). Following a significant F value, *post hoc* analysis (Newman-Keuls) were performed for assessing specific group comparisons. Calculations were performed using the BMDP statistical package.

Table 1. Effects of acute i.p. administration of the cannabinoid receptor antagonist SR 141716A (3 mg kg^{-1}) on defensive behaviour under novelty conditions

Treatment	Vehicle	SR 141716A[a]
Emergence latency (s)	246.3 ± 113.3	600 ± 0*
Time spent in (s)	515.3 ± 53	600 ± 0
Entries	3.5 ± 1.3	1.0 ± 0*
Mean time per entry (s)	300.7 ± 101.3	600 ± 0*
Crossings	35.2 ± 23.3	0.0 ± 0
Rearings	8.8 ± 6.3	0.0 ± 0

Values are means ± s.e.m. of six animals per group.
*$p < 0.05$, Newman-Keuls *vs* vehicle-treated animals.
[a]None of the animals exposed to SR 141716A emerged to explore the open field of the defensive withdrawal test.

Results

Effects of acute administration of SR 141716A on defensive withdrawal under familiar conditions: Acute administration of SR 141716A induced a dose-dependent increase in the emergence latency to the open field ($F(3,38) = 5.72$, $p < 0.005$, Fig. 1A), which was significant at the 3 mg kg^{-1} dose 30 min after injection of the drug. This dose also increased both the total time spent in the chamber ($F(3,38) = 4.85$, $p < 0.005$; data not shown) and the mean time spent in the chamber per entry ($F(3,38) = 4.55$, $p < 0.01$, Fig. 1B). The animals exposed to this dose of the CB_1 antagonist also exhibited less number of crossings ($F(3,38) = 5.45$, $p < 0.005$) and rearings ($F(3,38) = 4.85$, $p < 0.006$) performed outside the chamber (Fig. 1C,D).

Effects of SR 141716A on the elevated plus-maze and the defensive withdrawal under novelty conditions: Acute treatment with the CB_1 antagonist SR 141716A (3 mg kg^{-1}) reduced both the proportion of time spent by the animals and the number of entries in the exposed arms of the elevated plus-maze ($F(1,32) = 4.92$, $p < 0.04$ and $F(1,32) = 4.22$, $p < 0.05$, respectively, Fig. 2A,B). The global activity (total arm entries) was not affected as a result of the administration of the cannabinoid antagonist ($F(1,32) = 0.15$, $p = 0.69$, n.s.). This dose also suppressed exploratory behaviour in the defensive withdrawal test under novelty conditions (Table 1).

Effects of the treatment with SR 141716A on plasma hormone levels: Acute administration of SR 141716A did not affect plasma levels of ACTH ($F(1,16) = 0.03$, $p = 0.86$, n.s.), corticosterone ($F(1,20) = 2.37$, $p = 0.14$, n.s.) or prolactin ($F(1,16) = 1.17$, $p = 0.29$, n.s.).

Effects of SR 141716A on locomotor activity: The acute administration of SR 141716A did not modify the pattern of locomotor activity displayed by the animals in the photocell cages ($F(1,17) = 0.22$, $p = 0.64$, n.s.). Animals of both groups displayed the same pattern of habituation to the photocell cages, as reflected in the progressive decrease of locomotor activity ($F(1,17) = 10.6$, $p < 0.005$).

Effects of SR 141716A on cannabinoid withdrawal-related behaviours: The acute administration of SR 141716A (3 mg kg^{-1}) did not affect the time spent grooming ($F(1,10) = 0.04$, $p = 0.84$, n.s.) or the

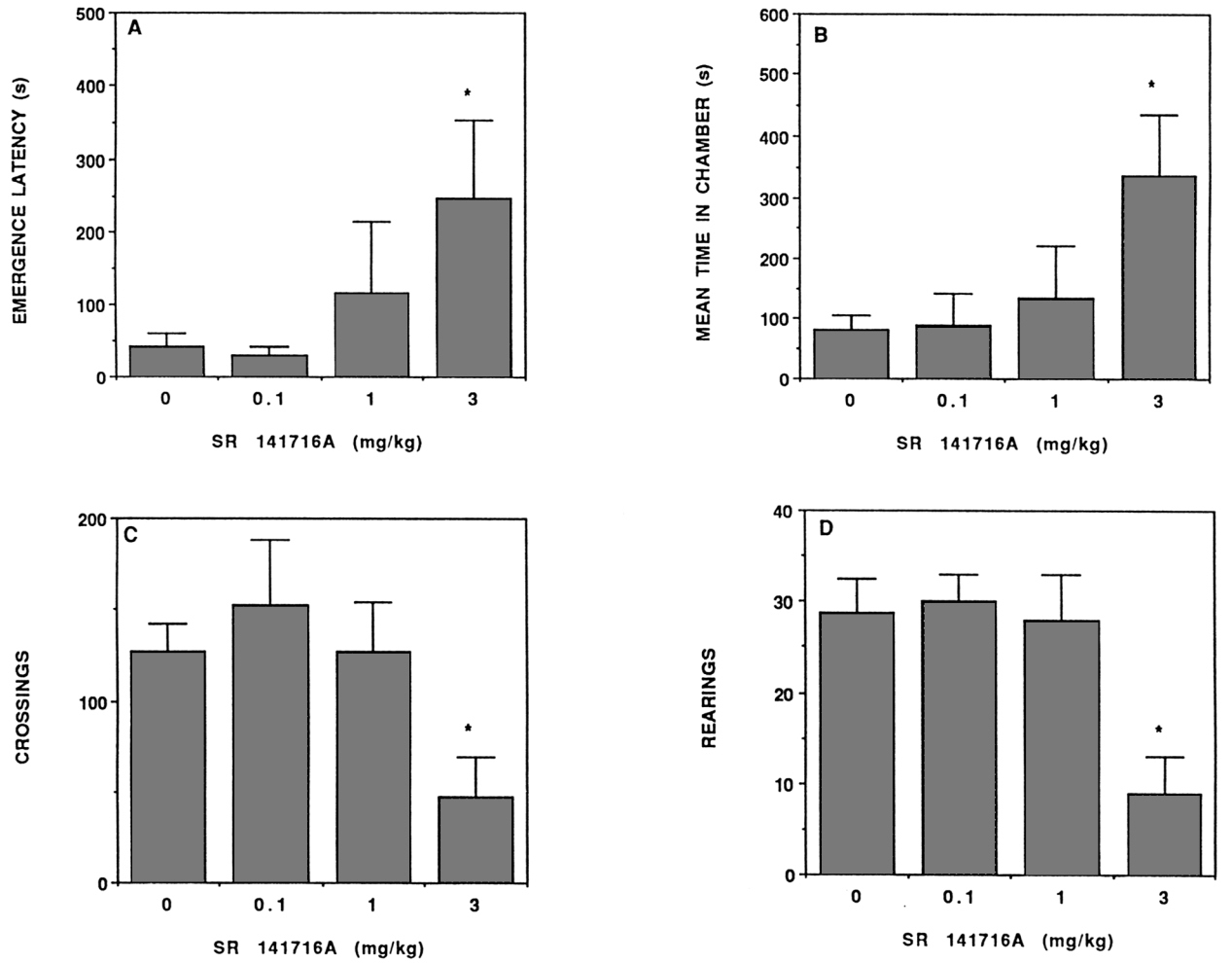

FIG. 1. Effects of acute i.p. administration of the cannabinoid receptor antagonist SR 141716A on defensive behaviour under familiar conditions. Rats were habituated to the environment for 10 min 24 h before the test. Values are means ± s.e.m. of 9–12 animals per group. *$p <$ 0.05 *vs* vehicle-treated animals, Newman-Keuls test.

Table 2. Plasma levels of corticosterone, adrenocorticotropic hormone (ACTH) and prolactin (PRL) 60 min after acute exposure to either vehicle or SR 141716A (3 mg kg^{-1})

Treatment	Vehicle	SR 141716A
Corticosterone (ng ml^{-1})	241.4 ± 48.9	317.5 ± 42.9
ACTH (pg ml^{-1})	98.6 ± 30	71.4 ± 30.4
PRL (ng ml^{-1})	5.9 ± 2.0	3.8 ± 0.7

Details in the text. Values are means ± s.e.m. of 8–12 determinations per group.

Table 3. Cannabinoid withdrawal-related behaviours observed after acute administration of either vehicle or the CB_1 receptor antagonist SR 141716A (3 mg kg^{-1})

Treatment	Vehicle	SR 141716A
Time spent grooming (s)	112.3 ± 52.4	124.3 ± 26.5
Rearings	82.5 ± 7.5	41.3 ± 15.1*
Wet dog shakes	1.2 ± 0.5	1.3 ± 0.8
Facial rubbings	1.1 ± 0.3	3.2 ± 0.5*
Scratching sequences	5.2 ± 2.8	30.3 ± 9.9*
Abdominal stretchings	0 ± 0	10.0 ± 3.7*

Details in the text. Values are means ± s.e.m. of six determinations per group.
*$p < 0.05$ *vs* vehicle group.

number of wet dog shakes ($F(1,10) = 0.03$, $p = 0.86$, n.s.) scored during the first 60 min post-treatment. However SR 141716A treatment reduced the number of rearings ($F(1,10) = 5.9$, $p < 0.04$) and increased the number of facial rubbings ($F(1,10) = 8.8$, $p < 0.02$), scratching sequences ($F(1,10) = 5.6$, $p < 0.04$) and abdominal stretchings ($F(1,10) = 7.2$, $p < 0.03$) observed.

Discussion

The present study provides the first evidence for the presence of an endogenous cannabinoid tone regulating emotional responses in rats. The acute administration of SR 141716A, the first CB_1 receptor antagonist described,[13] induced anxiety-like responses in both the defensive withdrawal test and the elevated plus-maze test. The effect was observed at a dose of 3 mg kg^{-1}. This dose has been previously found to increase the population activity of ascending nigrostriatal dopaminergic neurones,[14] to increase the time spent in wakefulness by decreasing the frequency of

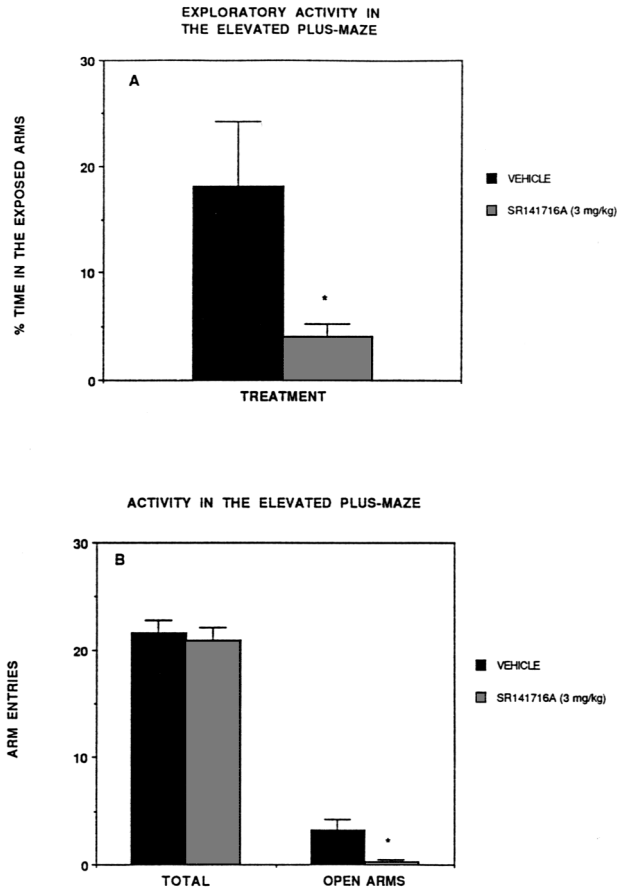

FIG. 2. Effects of SR 141716A on the behaviour displayed in the elevated plus-maze. Values are means ± s.e.m. of 9–12 animals per group. $*p < 0.05$ *vs* vehicle-treated animals, Newman-Keuls test.

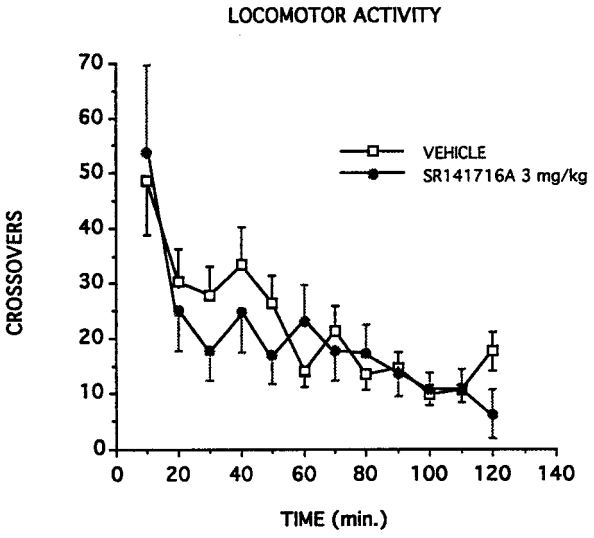

FIG. 3. Locomotor activity displayed by animals receiving an acute i.p. dose of either vehicle or the cannabinoid antagonist SR 141716A (3 mg kg^{-1}). Values are means of the crossovers registered at 10 min intervals of 9–10 animals per group. $*p < 0.05$ *vs* vehicle-treated animals, Newman-Keuls test.

both slow-wave sleep and rapid eye movement sleep,[15] and to antagonize the discriminative stimulus properties of natural and synthetic cannabinoids[16] in rats. However, this dose neither influences the pattern of horizontal locomotor activity nor the total activity in the elevated plus-maze. Additionally, SR 141716A administration did not alter the secretion of stress-related hormones, such as ACTH, corticosterone and prolactin. However, several cannabinoid withdrawal-related behaviours[18] were observed after the acute administration of SR 141716A, suggesting that there is an endogenous cannabinoid tone regulating the organization of motor sequences, but not locomotor activity. These effects contrast with the well described actions of CB_1 agonists, which induce defensive responses in both anxiety tests,[4–6] but also modify dramatically locomotor activity,[6] increasing the activity of the HPA axis, and depressing plasma prolactin levels.[9,10] These findings suggest that the recently described arousal enhancing properties of CB_1 receptor blockade [15]are associated with anxiety-like reactions, as measured in two behavioural models of anxiety. These anxiogenic properties seemed to be independent of the HPA axis, suggesting a differential origin for the behavioral and endocrine responses. This hypothesis is supported by the fact that CB_1 agonists are able of displaying both anxiogenic and anxiolytic properties,[5] depending on the dose and context of study (novelty *vs* familiar conditions), but they do not display biphasic actions on the HPA axis since they are dose-dependent activators of the adrenal response.[9,10,12] In this regard, it has been recently reported that acute administration of low doses of the highly potent synthetic cannabinoid HU-210 (4 μg kg^{-1}) induced anxiolytic responses in rats, abolishing the behavioural response to novelty in the defensive withdrawal test, without activating the HPA axis.[5] By contrast, higher doses of this potent CB_1 agonist (20 and 100 μg kg^{-1}) resulted in the opposite pattern, inducing anxiety reactions under novelty and familiar conditions, and increasing plasma corticosterone levels. These actions support the proposed differential actuation of cannabinoids on anxiety and the HPA activity.[5]

The particular behavioural and endocrine profile of SR 141716A might be explained as a result of the anatomical distribution of CB_1 receptors in stress-related brain areas.[8] In addition to the dense presence of CB_1 receptors in basal ganglia, cerebellum and hippocampus, these receptors are present in the central nucleus of the amygdala, the paraventricular nucleus of the hypothalamus and limbic cortex (prefrontal and cingulate).[8] Moreover, these areas are also activated after acute administration of cannabinoids, as mapped using the appearance of immunoreactivity for the early gene c-*fos*.[22] Since the central

amygdaloid nucleus is a key station for the behavioural response to stressful stimuli, whereas the paraventricular nucleus is the counterpart for orchestrating the neuroendocrine response to stress,[23] it is possible that the behavioural and endocrine actions described for CB_1 receptors agonists and antagonists might be elicited separately in these brain nuclei. The balance between the actuation on CB_1 receptors present in the paraventricular nucleus, and those present in the central nucleus of the amygdala and its efferents nuclei (accumbens shell and bed nucleus of the stria terminalis) might result in anxiogenic or anxiolytic responses associated or not to an increased adrenal response. This hypothesis is supported by the finding that corticotropin-releasing factor (CRF), one of the neuropeptides present in the above described brain nuclei, and which has been proposed to have a prominent role in the onset of anxiety reactions and in the control of anterior pituitary ACTH release,[23] participate in CB_1 agonists-induced anxiety-like responses.[5] In addition, recent studies (Rodríguez de Fonseca *et al*, submitted) suggest that acute administration of SR 141716A results in a dose-dependent moderate activation of brain nuclei related to the stress response, as mapped using FOS immunohystochemistry.

Conclusion

In summary, the CB_1 receptor antagonist SR 141716A elicited defensive responses in rats in two behavioural models of anxiety, suggesting the existence of an endogenous cannabinoid tone involved in the regulation of the emotional responses. This endogenous tone did not seem to be extensive to the HPA axis.

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References

1. Hollister LE. *Pharmacol Rev* **38**, 1–20 (1986).
2. Fishman MW, Rosenbaum JF, Yabusaki DI *et al. Res Commun Subst Abuse* **9**, 219–226 (1988).
3. Zuardi AW, Shirakawa I, Finkelfarb E *et al. Psychopharmacology* **76**, 245–250 (1982).
4. Onaivi ES, Green MR and Martin BR. *J Pharmacol Exp Ther* **253**, 1002–1009 (1990).
5. Rodríguez de Fonseca F, Rubio P, Menzaghi F *et al. J Pharmacol Exp Ther* **276**, 56–64 (1996).
6. Navarro M, Fernández-Ruiz JJ, de Miguel *et al. Behav Brain. Res* **5**, 37–46 (1993).
7. Devane WA, Dysarz FA, Johnson MR *et al. Mol Pharmacol* **34**, 605–613 (1988).
8. Herkenham M, Lynn AB, Johnson MR *et al. J Neurosci* **11**, 563–583 (1991).
9. Rodríguez de Fonseca F, Fernández-Ruiz JJ, Eldridge JC *et al. Pharmacol Biochem Behav* **40**, 593–598 (1991).
10. Rodríguez de Fonseca F, Villanúa MA, Muñoz RM *et al. Neuroendocrinology* **61**, 714–721 (1995).
11. Devane WA, Hanus L, Breuer A *et al. Science* **258**, 1946–1949 (1992).
12. Weidenfeld J, Feldman S and Mechoulam R. *Neuroendocrinology* **59**, 110–112 (1993).
13. Rinaldi-Carmona M, Barth F, Héaulme M *et al. FEBS Lett* **650**, 240–244 (1994).
14. Gueudet C, Santucci V, Rinaldi-Carmona M *et al. NeuroReport* **6**, 1293–1297 (1995).
15. Santucci V, Storme J-J, Soubrié P *et al. Life Sci* **6**, PL 103–110 (1996).
16. Perio A, Rinaldi-Carmona M, Maruani J *et al. Behav Pharmacol* **7**, 65–71 (1996).
17. Compton DR, Aceto MD, Lowe J *et al. J Pharmacol Exp Ther* **277**, 586–594 (1996).
18. Tsou K, Patrick SL and Walker JM. *Eur J Pharmacol* **280**, R 13–15 (1995)
19. Takahashi LK, Kalin NH, Van de Burgt JA *et al. Behav Neurosci* **103**, 648–654 (1989).
20. Pellow S, Chopin P, File SE *et al. J Neurosci Methods* **14**, 149[–]167 (1985).
21. Rubio P, Rodríguez de Fonseca F, Muñoz RM *et al. Life Sci* **56**, 2169–2176 (1995).
22. Herkenham M and Brady LS. *Soc Neurosci Abstr* **20**, 1676 (1994).
23. Koob GF, Heinrichs SC, Menzaghi F *et al. Semin Neurosci* **6**, 221–229, (1994).

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