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SR141716A induces in rats a behavioral pattern opposite to that of CB1 receptor agonists

Barbara COSTA[1], Mariapia COLLEONI (*Department of Pharmacology, Chemotherapy and Medical Toxicology, University of Milan, via Vanvitelli 32, Milan 20129, Italy*)

KEY WORDS SR141716A; cannabinoids; stereotyped behavior; pain measurement

ABSTRACT

AIM: To examine the acute actions of the CB1 cannabinoid receptor antagonist SR141716A [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] on typical behavioral pattern of psychoactive cannabinoids in rats. **METHODS:** At different time after injection the tail-flick response latency, the rectal temperature, the locomotor activity, and the immobility on a ring as well as the numbers of rears, self-grooming episodes (lasting 5 s), and fecal pellets were measured. **RESULTS:** Acute administration of SR141716A (3 mg/kg ip) induced a significant increase in horizontal locomotor activity assayed by an activity meter, in stereotypic activity (such as rearing and self-grooming) and in defecation, and a decrease in nociceptive threshold recorded as tail-flick latency. This dose had no effect on ring immobility and did not change the body temperature. **CONCLUSION:** These results demonstrate that this cannabinoid antagonist itself was inducing behavior opposite to that of CB1 receptor agonists.

INTRODUCTION

The recent identification and cloning of two G-protein-coupled cannabinoid receptors CB1 and CB2[1,2], the anatomical distribution of CB1 receptors in the CNS[3-5] and in certain peripheral tissues[6,7], and CB2 receptors only outside the central nervous system, the discovery of putative endogenous cannabinoid ligands, anandamide and 2-arachidonylglycerol[8-10] have led to the emergence of a new neurochemical system. Although much is known about the central effects of exogenously applied cannabinoids, the function of endogenous cannabinoid systems remains largely to be elucidated. In this context, the discovery of a potent and selective CB1 receptor antagonist, SR141716A[11], has opened new possibilities for the investigation of the functional role of the receptor-endocannabinoid complex in neuronal regulation. SR141716A, a pyrazole derivative, binds with higher affinity to CB1 receptors in rat brain than to CB2 receptors and fully reverses most of the behavioral, electrophysiologic, and biochemical effects of the cannabinomimetic compounds[11-13]. SR141716A was previously described as being devoid of any "intrinsic" activity in several mouse and rat pharmacological measures; afterwards, it has been found to possess a potent intrinsic activity on several physiological or behavioral parameters[14].

This present study was designed to explore the potential intrinsic activity on rat behavior of SR141716A at the dose of 3 mg/kg ip. This pharmacological dose was previously reported by us to block the behavioral effects of the endocannabinoid anandamide (20 mg/kg, ip) in various behavioral rat models such as hypothermia, immobility on a ring, and inhibition of motor behavior, when the antagonist was ip administered 30 min before anandamide[15].

MATERIALS AND METHODS

Drugs SR141716A [*N*-(piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] was kindly supplied by Sanofi Rechercbe, Montpellier, France. It was dissolved in

[1] Correspondence to Dr Barbara COSTA.
Phn 39-02-738-5568. Fax 39-02-7000-2270.
E-mail Colleoni@imiucca.csi.unimi.it
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Tween 80, Me_2SO, and distilled water (1:2:7) and administered ip in 1 mL/kg body weight.

Animals Male Sprague-Dawley CD rats (Charles River, Calco, Lecco, Italy), weighing (186.5 ± 7.77) g, in the 172 – 192 g range, 42-d old, were kept in a temperature-controlled (21 ℃) environment on a 12-h light/dark cycle, with free access to food and water, except during behavioral observations. They were handled daily before the experiments. All the procedures were carried out according to the local ethical regulations for animal research (Permission N° 16/1994-A).

Rats were acclimatized to the evaluation room (21 ℃) for 1 h before treatment; then they were given one ip injection of either vehicle (n = 7) or SR141716A (3 mg/kg) (n = 5) and observed for 60 min to measure the behavioral effects. All the rats were singly evaluated in a tetrad of assays, which together have been shown to be highly predictive of cannabinoid-induced activity[16] in mice: the tail-flick response latency was measured at 5, 15, 30, 45, and 60 min after the injection; the rectal temperature at 15, 30, 45, and 60 min; the locomotor activity for 5 min at 20 to 25 and at 50 to 55 min and the immobility on a ring for 5 min at 25 to 30 and at 55 to 60 min. The numbers of rears, self-grooming episodes (lasting 5 s), and fecal pellets were counted at 0 to 30 and at 30 to 60 min after injection.

Behavioral testing The nociceptive threshold was assessed by tail-flick test[17] and was quantified in terms of area under the curve (AUC), expressed as the response latency (s) to radiant heat (the intensity of the heat stimulus was fixed to give control latencies of 7 – 8 s) at 0 to 30 min and at 30 to 60 min. Rectal temperature was recorded with a rectal thermocouple inserted 5 cm into the anus and connected to a digital monitor (Ellab, Roedovre, Denmark). Rats were trained to the measure of the body temperature before the experiment. Spontaneous locomotor activity was assessed as the number of crossovers for a 5-min period, using an activity meter (Animex, LKB, USA; 40 cm × 25 cm divided into six magnetic solenoids of equal size on two lines). After each animal testing, the apparatus was cleaned with an odoriferous solution to prevent olfactory cues from affecting the behavior of subsequently tested rats. Ring immobility time(s) was assessed for 5 min on a horizontal wire ring, 12 cm in diameter, attached to a stand at a height of 38 cm[18]. The criterion for immobility was the absence of all voluntary movements, excluding respiration but including those of the whiskers. Also for this behavior evaluation, rats were trained before the experiment.

Statistical methods Data were expressed as $\bar{x} \pm s$ (standard deviation) of 5 – 7 animals; comparison between SR141716A and respective vehicle-treated rats was done using t test.

RESULTS

The effects of SR141716A on locomotor activity, ring immobility, body temperature, and nociceptive threshold are shown in Fig 1. Administration of the specific brain cannabinoid receptor antagonist (ip) had no significant effect on body temperature and ring immobility time at all evaluation times. It produced a significant increase in locomotor activity in 5 min at 20 to 25 min (+194 %; t = 10.44) and a significant decrease in the AUC of the tail-flick test at 0 to 30 min after injection (−31.6 %; t = 3.69). During the 30 min after SR141716A administration, there was an increase in stereotypic activity, such as rearing (+60 %; t = 4.47) and grooming, particularly scratching and scraping (+232 %; t = 4.00); while at 30 to 60 min after administration, only an increase in grooming was shown. However, it was smaller (+85 %; t = 3.24) than that at 0 to 30 min (Fig 2). Within the period of 0 to 30 min after administration there was also a significant increase (+104 %; t = 3.82) in number of fecal pellets, some of which almost diarrhoeic (Fig 2).

DISCUSSION

SR141716A was previously described as being devoid of any intrinsic activity in several mouse and rat pharmacological measures, while we have shown that the cannabinoid CB1 receptor antagonist by itself produced decreasing effects in nociceptive thermal threshold and increasing effects in locomotor activity, spontaneous non-ambulatory activities (such as grooming and rearing), and defecation. These intrinsic effects lasted 30 min, except for grooming episodes that were increased during second half hour following the SR141716A administration. There are

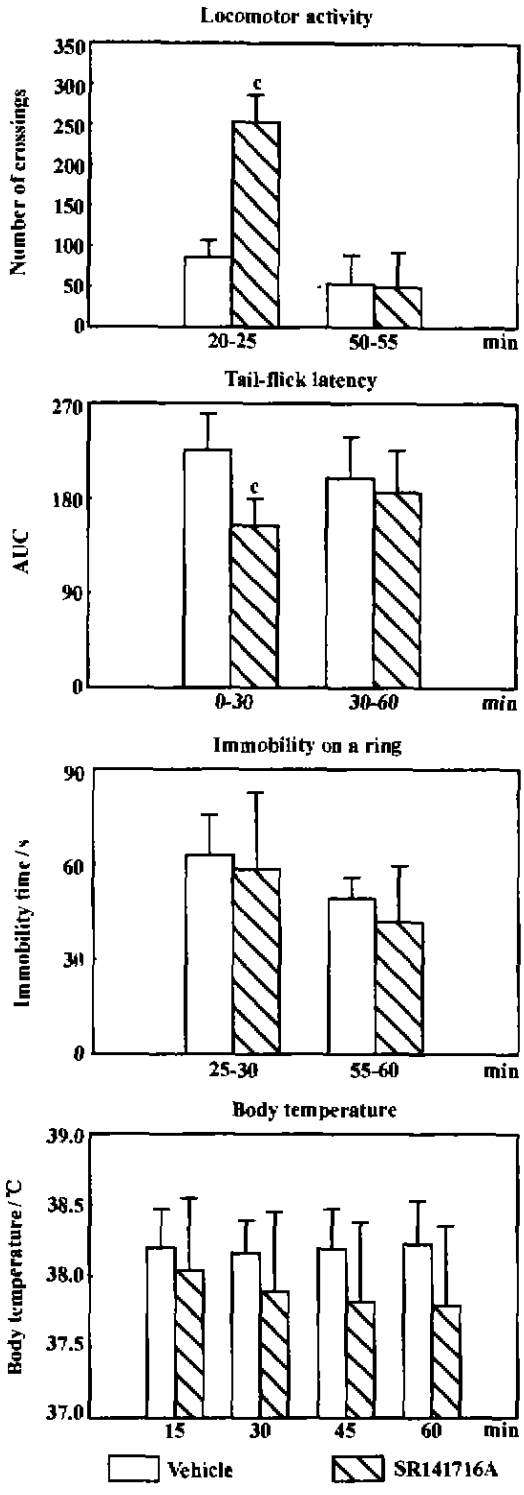

Fig 1. Effects of SR141716A (3 mg/kg ip) on locomotor activity, tail-flick latency, immobility on a ring, and body temperature in rats. $n = 5 - 7$ rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* vehicle.

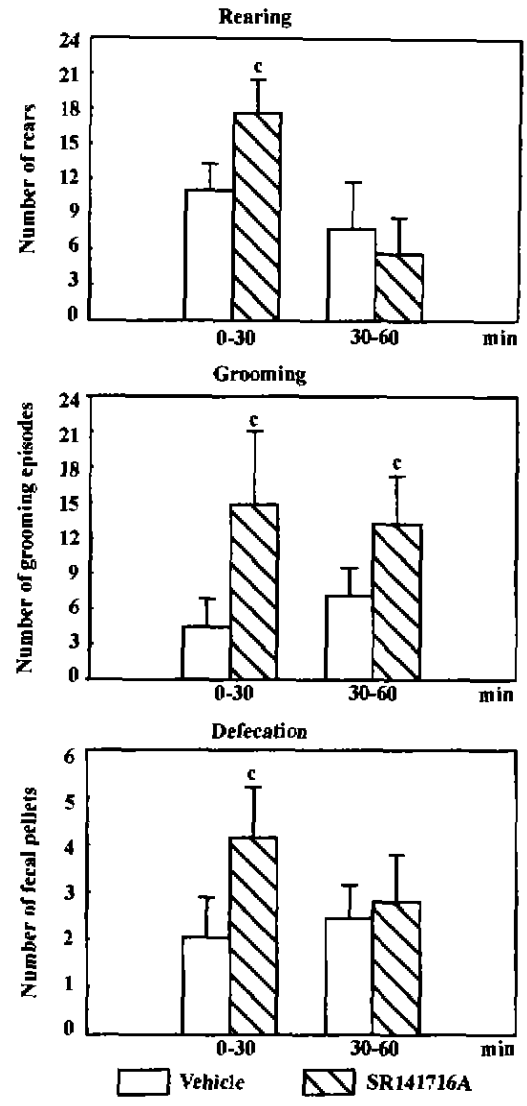

Fig 2. Effects of SR141716A (3 mg/kg ip) on number of rearing, grooming, and fecal pellets in rats. $n = 5 - 7$ rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* vehicle.

previous reports that SR141716A, when administered alone, produced a significant increase in mice gastrointestinal transit and defecation and in the intestinal fluid volume in rat[19-21] and that it produced a significant increase in acetylcholine release from the myenteric plexus of the guinea-pig small intestine[22] and that it stimulated the amplitude of cholinergic and electrically-evoked guinea-pig ileal contractions[23,24]. Our results and the results of the previous studies demonstrate that cannabinoid CB1 receptors are present in small intestine and they are involved in the regulation of the intestinal propulsion. Compton *et al* previously

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have reported that SR141716A produced locomotor stimulation in a dose-responsive fashion with doses from 3 to 20 mg/kg, when administered iv in mice[12]. Our results together with those of Compton *et al*[12] suggest the presence of an inhibitory endogenous cannabinoid tone regulating spontaneous locomotor activity.

In addition, Richardson *et al* have shown that injection of SR141716A in otherwise untreated mice produced hyperalgesia in the hot plate test[25,26]; Meng *et al*[27] found that SR141716A (0.5 mg/kg) administration iv decreased tail-flick latencies significantly in rats; Herzberg *et al*[28] found that ip injection of this compound increased thermal hyperalgesia and mechanical allodynia in untreated rats with chronic constriction injury of the sciatic nerve; Strangman *et al*[29] found that SR141716A enhanced responsiveness in the rat formalin test, when injected prior to or subsequent, to formalin injection. These previous results and our results suggest that the cannabinoid system tonically regulates thermal nociceptive thresholds. Furthermore, the absence of this regulation results in hyperalgesia, suggesting that hypoactivity of this system may be involved in certain types of chronic pain. As well as us, Navarro *et al* demonstrated that SR141716A at 3 mg/kg ip induced in rats cannabinoid withdrawal-like behavioral symptoms, such as grooming, rearings, facial rubbings, scratching sequences, and abdominal stretching[30], suggesting the existence of endogenous cannabinoid tone involved in the regulation of the emotional responses.

Our results suggest that SR141716A inhibits the endogenous cannabinoid system, because it produced all the effects that were opposite to those produced by endocannabinoid agonists. It is also possible that SR141716A could act as an inverse agonist[31], according to Milligan *et al*[32] and to Leff[33]; most specifically SR141716A could produce effects in the absence of other drugs, because it binds preferentially to the receptors in the uncoupled state from the effector system, thereby shifting the equilibrium away from those in the precoupled state and hence it would be expected to produce an attenuation in inhibiting activity of endocannabinoids[22]. Some studies *in vivo* and *in vitro* have shown that SR141716A, when given alone, is an inverse agonist. In fact, Terranova *et al*[34] showed that SR141716A reduced memory deficits in aged rats and improved social recognition in adult rats. In Chinese hamster ovary cells expressing the human cannabinoid CB1 receptor SR141716A produced a decrease in basal incorporation and binding of [^{35}S]GTPγS[31,35] and blocked the activities of mitogen-activated protein kinase and adenylyl cyclase[36]. In neurons both of superior cervical ganglion heterologously expressing the rat CB1 receptor and of pelvic ganglion with native cannabinoid receptors it increased voltage-dependent Ca^{2+} currents[37].

In conclusion, the use of the highly selective CB1 antagonist SR141716A provides further support for the role of endogenous cannabinoids as neurotransmitter or neuromodulator in many physiological functions. Cannabinoid receptor antagonists as new class of therapeutic agents, may decrease the background tone resulting from the release of endocannabinoids and could be useful in the treatment of pathological conditions with motor deficits (multiple sclerosis, spinal injury) and of gastrointestinal motility and emotional disorders in humans.

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SR141716A 诱导大鼠产生与 CB1 受体激动剂相反的行为模式

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Barbara COSTA[1], Mariapia COLLEONI

(*Department of Pharmacology, Chemotherapy and Medical Toxicology, University of Milan, via Vanvitelli 32, Milan 20129, Italy*)

关键词 SR141716A; 大麻酚类; 刻板行为; 疼痛测定

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