Psychopharmacology (1998) 135 : 324–332

ORIGINAL INVESTIGATION

Frédérique Chaperon · Philippe Soubrié
Alain J. Puech · Marie-Hélène Thiébot

Involvement of central cannabinoid (CB_1) receptors in the establishment of place conditioning in rats

Received: 14 March 1997 / Final version: 28 July 1997

Abstract The involvement of cannabinoid processes in positive reinforcement was studied using an unbiased, one-compartment, conditioned place preference (CPP) procedure in rats. This was achieved by examining the ability of the selective antagonist of the CB_1 cannabinoid receptor subtype, SR 141716, to counteract the CPP supported by classical reinforcers. The acquisition of CPP induced by cocaine (2 mg/kg), morphine (4 mg/kg) and food (standard chow and sucrose pellets) was dose-dependently blocked by pre-pairing administration of SR 141716 (0.03–3 mg/kg). However, SR 141716 (up to 10 mg/kg) did not significantly counteract the expression of cocaine-induced CPP. On the other hand, the synthetic CB receptor agonist, WIN 55212-2 (0.3–1 mg/kg), established a robust place aversion (CPA), as already described with other agonists, and CPP was never observed, even at 100-fold lower doses. The aversive effect of WIN 55212-2 was reversed by SR 141716 (0.3–1 mg/kg), suggesting that it was accounted for by the stimulation of CB_1 receptors. These findings indicate that, on their own, CB receptor agonists are unable to generate the processes necessary to induce a pleasurable state in animals, as assessed in place conditioning procedures. Nevertheless, a cannabinoid link may be involved in the neurobiological events, allowing the perception of the rewarding value of various kinds of reinforcers. However, a permanent endogenous cannabinoid tone seems unlikely to be necessary to ensure the organism a basal hedonic level since, given alone, SR 141716 supported neither CPP nor CPA.

Key words Cannabinoid receptors · Cocaine · Food · Incentive learning · Morphine · Rat · Reward · SR 141716 · WIN 55212-2

F. Chaperon · M.H. Thiébot (✉)
INSERM U.288, Faculty of Medicine Pitié-Salpêtrière,
91 bd de l'Hôpital,
F-75634 Paris Cedex 13, France
Fax (+33) 140779596, e-mail: thiebot@idf.ext.jussieu.fr

A.J. Puech
Department of Pharmacology,
Faculty of Medicine Pitié-Salpêtrière, 91 boulevard de l'Hôpital,
F-75634 Paris Cedex 13, France

P. Soubrié
SANOFI Recherche, 371 rue du Professeur J. Blayac,
F-34184 Montpellier Cedex 04, France

Introduction

A number of drugs of abuse, such as cocaine, morphine and amphetamine, presumably derive their appetitive properties from their ability to activate brain reward circuits, and considerable evidence supports that this effect involves a direct or indirect stimulation of the mesolimbic dopaminergic (DA) system (Bozarth 1991). The bases of the addictive properties of marijuana (*Cannabis sativa*) and its derivatives remain to be clearly identified. Animal studies indicate that these compounds induce both rewarding and aversive effects. In rats, low doses of Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive component of cannabis, were self-administered (Takahashi and Singer 1979), facilitated intracranial self-stimulation in the medial forebrain bundle (Gardner et al. 1988) and supported conditioned place preference (CPP) (Lepore et al. 1995). In keeping with the hypothesis that positive reinforcing effects of drugs would be accounted for by an activation of DA transmission, such low doses of Δ^9-THC have been shown to increase DA outflow in reward-relevant brain areas such as the medial prefrontal cortex and the nucleus accumbens (Chen et al. 1990a,b). However, a number discrepant findings have also been reported. Thus, Castañeda et al. (1991) found that Δ^9-THC, in the same dose range, failed to affect either basal or amphetamine-induced stimulation of DA

release in the nucleus accumbens. Furthermore, self-administration of Δ^9-THC in animals has never been reproduced, and several former attempts to establish cannabinoids as reinforcers of self-administration behaviour were unsuccessful (Corcoran and Amit 1974; Harris et al. 1974; Leite and Carlini 1974). Cannabinoids induce anxiogenic-like effects in the elevated plus maze in rodents (Onaivi et al. 1990). Finally, several studies showed that the synthetic cannabinoid agonist CP 55,940, as well as Δ^9-THC (at slightly higher doses than those supporting CPP), could induce conditioned place avoidance (CPA) and taste aversion rather than CPP and/or appetitive effects (Lepore et al. 1995; Parker and Gillies 1995; McGregor et al. 1996; Sañudo-Peña et al. 1997).

Two types of cannabinoid receptors have been identified to date, the CB_1 receptor, essentially located in the central nervous system (Matsuda et al. 1990) and the CB_2 receptor which is found predominantly in peripheral tissues (Munro et al. 1993). Natural (Δ^9-THC) and synthetic (CP 55,940, WIN 55212-2) agonists, as well as the putative endogenous ligand, anandamide, bind to both CB_1 and CB_2 receptors (Munro et al. 1993; Howlett 1995). In contrast, the antagonist SR 141716 binds selectively and with high affinity to CB_1 receptors (Rinaldi-Carmona et al. 1994). As an antagonist, this drug blocked the responses elicited by Δ^9-THC or WIN 55212-2 in several tests, including ring-immobility (catalepsy), hypothermia and analgesia in rodents (Rinaldi-Carmona et al. 1994,1995; Compton et al. 1996). In addition, SR 141716 counteracted the discriminative stimulus cues of Δ^9-THC, WIN 55212-2 and CP 55,940, in monkeys and/or rats (Wiley et al. 1995a,b; Pério et al. 1996), and prevented the Δ^9-THC-induced deficit in radial maze performance in rats (Lichtman and Martin 1996).

SR 141716 was recently shown to reduce markedly and selectively sucrose- and ethanol-directed appetitive behaviour in rodents (Arnone et al. 1997). Therefore, it was interesting to use this antagonist as a tool to investigate whether cannabinoid processes would be implicated in positive reinforcement. For that purpose, the ability of SR 141716 to counteract CPP induced by the non-cannabinoid appetitive drugs, cocaine and morphine, and also by food, and the intrinsic motivational value of SR 141716, were studied in rats subjected to an unbiased place conditioning procedure. Such a paradigm, which allows the assessment of the perception of the motivational value of a reinforcer, is based upon the principle that animals would learn to approach or avoid environmental stimuli which have been repeatedly paired with rewarding or aversive events, respectively (see Carr et al. 1989). In addition, since some of the effects of CB receptor agonists have been found to follow biphasic dose-effect relationships, the ability of low and large doses of WIN 55212-2 to establish place conditioning were also investigated.

Materials and methods

Animals

The experiments were carried out on drug- and test-naive male Wistar AF rats (CERJ, Le Genest, France) weighing 240 ± 10 g at the beginning of the experiments. They were housed eight per cage under standard conditions (12 h light-dark cycle; room temperature 21°C) with free access to water in their home cage. One week prior to the beginning of the experiments, rats were food restricted (13 g/day of standard chow) until the end of the study, in order to homogenise the experimental conditions throughout the study, whether or not food was provided during place conditioning. On each of the 5 days prior to the first conditioning trial, rats were handled, weighed and habituated to the injection procedure. Experiments were performed in agreement with French ethical rules on animal care.

Place conditioning paradigm

Apparatus

The experiments were conducted as previously described (Guyon et al. 1993; Chaperon and Thiébot 1996). Briefly, animals were trained and tested in black wooden open fields ($76 \times 76 \times 50$ cm) located in a dimly lit room, supplied with continuous masking noise. The floor of each open field was covered with removable quadrants made from one of two textures, wire mesh or rough Plexiglas. The behaviour of the animals was videotaped using cameras mounted 200 cm above each open field. Control and recording equipment were situated in the adjacent room.

Experimental procedure

The general, unbiased, procedure consisted of two phases: conditioning and testing. Each rat was subjected to eight 30-min conditioning sessions (two sessions per day, unless otherwise specified) in one open field whose four floor quadrants were of the same texture. The drugs tested (or vehicle for the associated control groups) were administered before the "even" numbered sessions (i.e. 2, 4, 6 and 8) paired with one floor texture. Saline was injected (same route of administration, same pretreatement time) before the "odd" numbered sessions (i.e. 1, 3, 5 and 7) paired with the other floor texture. Drug-texture pairings were counterbalanced.

The day following the last conditioning session, the rats received a single 20-min test session in the open field whose floor was made up of two quadrants of the saline-paired texture and two quadrants of the drug-paired texture. The quadrants of the same texture were positioned diagonally opposite to each other. Rats were given no injection before the test session.

The time spent on each texture during the test session was scored from the videotapes by an experimenter blind to the previous pairing conditions. Rats were considered to be on a floor quadrant when their four paws were on that quadrant. Half of the time spent on the dividing lines was added to the total time spent on the drug-paired texture. The number of quadrants crossed during the test session was also recorded.

Experiment 1: ability of SR 141716 and WIN 55212-2 to support place conditioning

SR 141716 (0.3–3 mg/kg) or WIN 55212-2 (0.003–1 mg/kg), or their vehicle for the control groups, were administered 30 min or 15 min, respectively, before the "even" conditioning sessions which

took place on the afternoon. Saline was injected before the "odd", morning, sessions.

Experiment 2: interaction between SR 141716 and WIN 55212-2

WIN 55212-2 (0.3 mg/kg) was injected 15 min before the "even" conditioning sessions. SR 141716 (0.3–1 mg/kg), or its vehicle for the associated control group, was administered 15 min before WIN 55212-2. Rats of all groups were given vehicle and saline according to the same schedule before each "odd" session.

Experiment 3: effect of SR 141716 on the establishment of food-induced conditioned place preference

Sucrose pellets (45 mg Campden), and the usual rat chow and water were provided in the open field during the "even" conditioning sessions. SR 141716 (0.3–3 mg/kg), or vehicle for the control group, was injected 30 min before each conditioning session with food. Saline was administered before each "odd" session without food. The amount of sucrose pellets and usual food eaten by each animal during each session was measured separately. During the test session, food and water were never available in the open field.

Experiment 4: effects of SR 141716 on the establishment of cocaine- and morphine-induced conditioned place preference

Cocaine (2 mg/kg) or morphine (4 mg/kg) was administered immediately before the "even" conditioning sessions. SR 141716 (0.03–3 mg/kg), or its vehicle for the associated control group, was injected 30 min before cocaine or morphine. All rats were given vehicle and saline according to the same schedule before each "odd" conditioning session. For the experiments with cocaine only, animals were subjected to a single conditioning session per day (in order to limit tissue necrosis due to the cocaine-induced vasoconstriction). The doses of cocaine and morphine were chosen from pilot dose-range studies performed according to the same experimental design.

Experiment 5: effects of SR 141716 on the expression of cocaine-induced conditioned place preference

Animals were given cocaine (2 mg/kg) immediately before each "even" session and saline immediately before "odd" sessions (one conditioning session per day). SR 141716 (0.3–10 mg/kg), or its vehicle for the associated control group, was administered 30 min before the only test session.

Experiment 6: effects of SR 141716 on cocaine-induced stimulation of motor activity

Locomotor activity was measured by means of photoelectric actimeters. Test- and drug-naive rats, not food-deprived, were individually placed in transparent Plexiglas boxes ($37 \times 37 \times 17$ cm) crossed, 5 cm above the floor, by two horizontal light beams at 90° to each other, detected by photocells. Each time the rat crossed a beam, one movement was recorded. SR 141716, or its vehicle for the associated control group, was injected immediately before placement into the actimeter. Cocaine (4 mg/kg) or saline was administered 30 min later. The number of light beams crossed was then recorded for 30 and 60 min. The dose of cocaine was chosen from a pilot experiment as the lowest one which significantly enhanced rats motor activity.

Drugs

SR 141716 [*N*-piperidino-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methylpyrazole-3-carboxamide hydrochloride] (SANOFI Recherche, Montpellier, France) and WIN 55212-2 [(*R*)-4,5-dihydro-2-methyl-4-(4-morpholinylmethyl)-1-(1-naphtalenylcarbonyl)-6H-pyrrolo[3, 2, 1-ij]quinolin-6-one mesylate] (Reserch Biochemicals, Natick, USA) were suspended with Tween 80 in distilled water. Cocaine HCl and morphine HCl (Coopération Pharmaceutique Française, Melun, France) were dissolved in saline (0.9% NaCl). Drugs and respective vehicle for associated control groups were administered SC (or IP for SR 141716) in a volume of 5 ml/kg to independent groups of rats. The doses are expressed as the salt.

For several compounds, the complete range of doses was studied in the course of two or three independent experiments, which always included one associated control group. The performance of these groups of control or treated rats were pooled for the analysis and presentation of the results.

Statistical analysis

The results, expressed as mean (± SEM) time (s) spent on the drug- or drug+food-paired texture and number of quadrants crossed during the test session, were analysed by one-way analysis of variance (ANOVA) with drug treatment as independent factor. The mean quantities of sucrose and usual food consumed during the conditioning sessions (experiment 3) were analysed by two-way (session and treatment) ANOVA. Planed pairwise comparisons were made using two-tailed (drug effect) or one-tailed (drug × drug or food × drug interactions) Dunnett's *t*-test using the appropriate error variance term from the ANOVAs.

Results

Experiment 1: ability of SR 141716 and WIN 55212-2 to support place conditioning

Control rats (which received saline and vehicle before "odd" and "even" sessions, respectively) exhibited individual place preference but group means did not significantly differ from the chance level (600 s).

The time spent on the floor texture previously associated with SR 141716 was not significantly different from control level [$F(3,90) = 1.38$; NS] (Fig. 1). This result indicates that SR 141716 did not support conditioned place preference or place aversion. The number of quadrants crossed during the test session was not modified by pre-pairing administration of SR 141716 [$F(3,90) = 1.40$; NS] (Table 1).

WIN 55212-2 induced an overall change in time spent on the drug-paired floor texture [$F(6, 89) = 5.19$; $P < 0.0001$]. Subsequent analysis revealed that animals given 0.3 and 1 mg/kg WIN 55212-2 spent significantly less time than controls on the drug-associated texture ($t = 3.50$ and $t = 3.55$, respectively, $P < 0.01$), indicating that WIN 55212-2 produced conditioned place aversion (Fig. 1). The activity during the test session was reduced in animals previously given WIN 55212-2 at the only dose of 1 mg/kg (number of quadrants crossed: controls: 56 ± 5; 1 mg/kg: 39 ± 4; Student's $t = 2.65$; $P < 0.02$).

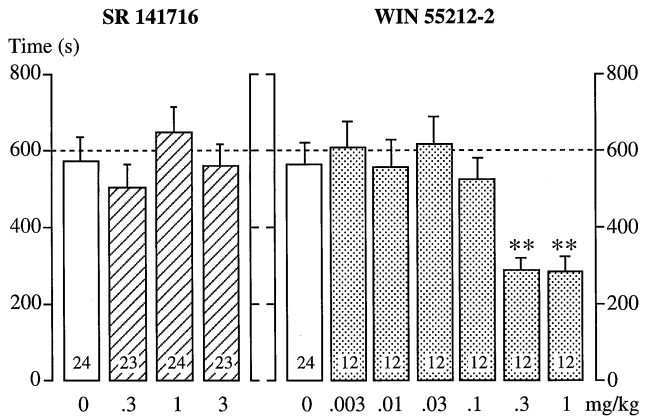

Fig. 1 Effects of SR 141716 and WIN 55212-2 on place conditioning (experiment 1). Histograms represent the time (mean + SEM) spent during the 20-min test session on the floor texture previously paired with SR 141716 or WIN 55212-2. The *horizontal dashed line* indicates the chance level (no preference). SR 141716 and WIN 55212-2 (or their vehicle) were injected IP 30 min and SC 15 min, respectively, before each of the four "even" conditioning sessions. Rats were drug-free during the test session (the number of rats per group is indicated in the histograms). $^{**}P < 0.01$ versus associated control group (Dunnett's *t*-test after ANOVA)

Experiment 2: interaction between SR 141716 and WIN 55212-2

Control rats spent 378 ± 40 s on the floor texture previously associated with WIN 55212-2 (0.3 mg/kg). This time was significantly shorter than the theoretical indifference level ($t = 4.79$, $P < 0.01$), indicating that rats developed an aversion for the WIN 55212-2-paired floor texture. Rats given SR 141716 exhibited an overall change in time spent on the WIN 55212-2-paired texture [$F(2,27) = 3.82$; $P = 0.03$]. Subsequent comparisons indicated that SR 141716 at 1 mg/kg lengthened significantly the time spent on the WIN 55212-2-paired texture ($t = 2.73$; $P < 0.01$) (Fig. 2). Motor activity during the test session was not significantly modified by the treatments [$F(2,27) = 0.95$; NS] (not shown).

Experiment 3: effect of SR 141716 on the establishment of food-induced conditioned place preference

Control rats spent 711 ± 51 s on the floor texture previously associated with food. This value, significantly above the theoretical 600-s chance level ($t = 2.17$, $P < 0.05$), indicates a preference for the food-paired floor texture. SR 141716 induced an overall change in time spent on this texture [$F(3,44) = 2.74$; $P = 0.05$]. Subsequent comparisons indicated that the time spent on the food-paired texture was significantly reduced in rats given 3 mg/kg SR 141716 ($t = 2.49$; $P < 0.05$); performance of this group did not differ from chance level ($t = 1.45$; NS) (Fig. 3). The number of quadrants crossed during the test session was not significantly modified by pre-conditioning administration of SR 141716 [$F(3,44) = 1.99$; NS] (not shown).

Table 1 Effects of SR 141716 on rats activity in the open field during the CPP test session (experiments 1 and 4), on spontaneous locomotion and on cocaine-induced stimulation of motor activity (experiment 6). CPP test session: SR 141716 and cocaine were administered daily, 30 min and immediately before the four "even" conditioning sessions, respectively; rats were given no injection before the test session. Actimetry: SR 141716 was injected immediately before rat placement into the actimeter and cocaine, 30 min later. Results are expressed as mean ± SEM

SR 141716 mg/kg IP	Cocaine mg/kg SC	*n*	CPP test session Number of quadrants crossed (20 min)	
0	–	24	56 ± 4	
0.3	–	23	51 ± 3	
1	–	24	46 ± 4	
3	–	23	53 ± 3	
0	2	36	68 ± 4	
0.03	2	12	66 ± 6	
0.1	2	12	74 ± 8	
0.3	2	36	65 ± 3	
1	2	23	64 ± 4	
3	2	23	67 ± 3	
			Actimetry number of light beams crossed	
			30–60 min	30–90 min
0	–	13	69 ± 17	86 ± 20
0.3	–	12	50 ± 10	68 ± 10
1	–	12	67 ± 22	89 ± 27
3	–	12	66 ± 22	85 ± 24
0	0	8	36 ± 18	39 ± 23
0	4	8	81 ± 16*	133 ± 32**
0.3	4	7	77 ± 27	122 ± 37
1	4	8	98 ± 22	197 ± 32
3	4	8	112 ± 36	235 ± 65

$^{*}P < 0.05$; $^{**}P < 0.02$; cocaine alone versus associated controls (vehicle + vehicle) (one-tailed Student's *t*-test)

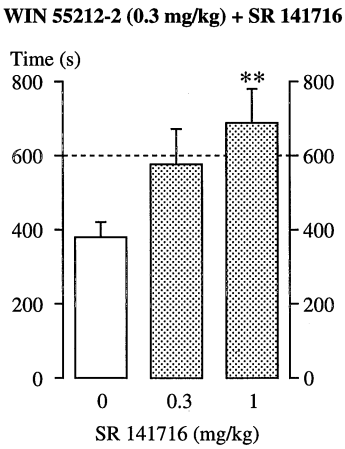

Fig. 2 Effects of SR 141716 on the establishments of WIN 55212-2-induced conditioned place aversion (experiment 2). Histograms represent the time (mean + SEM) spent during the 20-min test session on the floor texture previously paired with WIN 55212-2+SR 141716. The *horizontal dashed line* indicates the chance level. WIN 55212-2 (0.3 mg/kg) and SR 141716 (or its vehicle) were administered SC 15 min and IP 30 min , respectively, before the four "even" conditioning sessions. Rats were drug-free during the test session ($n = 10$/group). $^{**}P < 0.01$ versus WIN 55212-2 + vehicle (Dunnett's *t*-test after ANOVA)

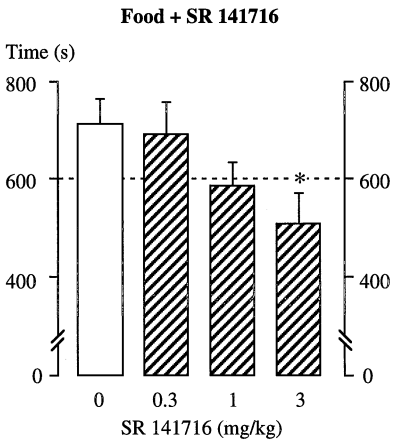

Fig. 3 Effects of SR 141716 on the establishments of food-induced conditioned place preference (experiment 3). Histograms represent the time (mean + SEM) spent during the 20-min test session on the floor texture previously paired with food + SR 141716. The *horizontal dashed line* indicates the chance level (no preference). SR 141716 or its vehicle was injected IP 30 min before each of the four "even" conditioning sessions with food. Rats were drug-free during the test session ($n = 12$/group). $P < 0.05$ versus vehicle controls (Dunnett's *t*-test after ANOVA)

The quantity of food consumed during the conditioning phase was significantly reduced by SR 141716. This was observed for both sucrose [dose effect: $F(3,44) = 9.92$; $P < 0.0001$; dose × session interaction: $F(9,132) = 3.48$; $P < 0.001$] and the usual food [dose effect: $F(3,44) = 3.28$; $P < 0.03$; dose × session interaction: $F(9,132) = 4.07$; $P < 0.0001$] (Table 2).

Experiment 4: effects of SR 141716 on the establishment of cocaine- and morphine-induced conditioned place preference

Rats spent 774 ± 30 s on the floor texture previously associated with cocaine (2 mg/kg). This value, significantly above the theoretical 600-s chance level ($t = 5.84$, $P < 0.01$), indicates a preference for the cocaine-paired floor texture. Pre-pairing administration of SR 141716 resulted in a significant reduction of the time spent on the cocaine-paired texture [$F(5,136) = 5.94$; $P < 0.001$]. Subsequent comparisons indicated that this dose-dependent effect was due to 0.1, 0.3, 1 and 3 mg/kg of SR 141716 which significantly shortened the time spent on the cocaine-paired floor texture ($t = 2.52$; $P < 0.05$; $t = 3.27$; $t = 3.34$ and $t = 5.16$; $P < 0.01$, respectively). In addition, this time was significantly below the chance level in rats given 3 mg/kg of SR 141716 ($t = 2.61$; $P < 0.01$) (Fig. 4). The number of quadrants crossed during the test session by rats previously given cocaine alone did not differ from control performance. SR 141716 co-administered with cocaine did not modify rats motor activity [$F(5,136) = 0.45$; NS] (Table 1).

Morphine (4 mg/kg) induced conditioned place preference as indicated by the time spent on the morphine-paired floor texture (829 ± 28 s) significantly above the chance level ($t = 6.95$; $P < 0.01$). This effect was modified by SR 141716 [$F(5,101) = 2.79$; $P = 0.02$]. Pairwise comparisons revealed that rats given 0.1 and 0.3 mg/kg SR 141716 spent significantly less time than controls on the morphine-paired texture ($t = 2.40$; $P < 0.05$ and $t = 3.25$; $P < 0.01$, respectively) (Fig. 4). The number of quadrants crossed by rats previously given SR 141716 plus morphine did not differ from morphine alone [$F(5,101) = 0.93$; NS] (not shown).

These results indicate that SR 141716 impaired the *establishment* of cocaine- and morphine-induced CPP.

Experiment 5: effects of SR 141716 on the expression of the cocaine-induced conditioned place preference

During the test session, rats spent 692 ± 36 s on the floor previously paired with cocaine (2 mg/kg). This value was significantly above the theoretical 600-s chance level ($t = 2.26$; $P < 0.05$). SR 141716, administered as a single injection before the test session, did not significantly modify the time spent on the texture previously paired with cocaine, i.e. failed to affect the expression of cocaine-induced CPP [$F(4, 78 = 0.49$; NS] (Fig. 5). However, the time spent on the cocaine-paired texture by rats given SR 141716 (3 mg/kg) did not differ from the chance level ($t = 0.18$; NS). SR 141716 induced an overall modification of the number of quadrants crossed during the test session [$F(4,78) = 6.30$; $P < 0.001$]. Pairwise comparisons revealed that this was

Table 2 Effects of SR 141716 on sucrose and usual food intake during the four food-texture pairing sessions (experiment 3). SR 141716 was injected 30 min before each 30-min conditioning session with food (n = 12/group). The results of ANOVAs calculated separately for each food consumed during each session are indicated in the table

mg/kg IP	Mean (± SEM) food intake (g) during conditioning sessions			
	1	2	3	4
Sucrose				
Vehicle	1.0 ± 0.2	2.2 ± 0.3	2.9 ± 0.4	3.1 ± 0.4
0.3	1.1 ± 0.2	1.6 ± 0.2	1.6 ± 0.3*	2.1 ± 0.2
1	0.6 ± 0.1	1.0 ± 0.2**	1.3 ± 0.3**	1.6 ± 0.2**
3	0.7 ± 0.2	0.5 ± 0.2**	0.8 ± 0.3**	1.0 ± 0.4**
F(3,44)	=1.16; NS	=9.92; $P < 0.001$	=7.98; $P < 0.001$	=7.93; $P < 0.001$
Usual food				
Vehicle	3.4 ± 0.3	3.3 ± 0.3	2.8 ± 0.2	2.9 ± 0.2
0.3	3.1 ± 0.4	2.9 ± 0.3	2.8 ± 0.3	3.3 ± 0.2
1	1.8 ± 0.3**	2.3 ± 0.3*	2.2 ± 0.4	2.9 ± 0.3
3	1.8 ± 0.3**	1.9 ± 0.2**	3.3 ± 0.3	2.9 ± 0.3
F(3,44)	= 6.26; $P < 0.002$	= 5.07; $P < 0.005$	= 1.94; NS	= 0.56; NS

*$P < 0.05$; **$P < 0.01$ versus associated vehicle control group (Dunnett's t-test)

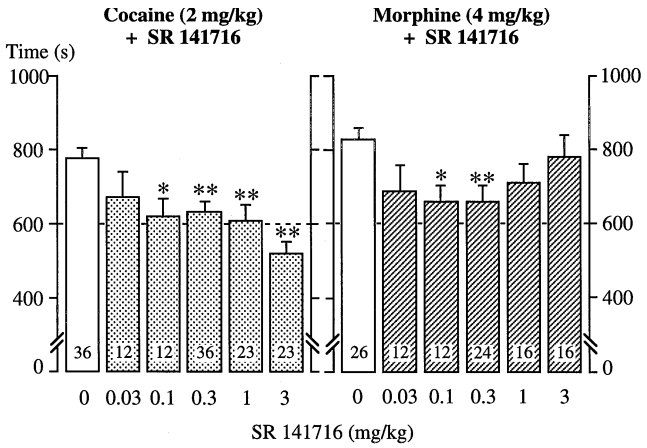

Fig. 4 Effects of SR 141716 on the establishment of conditioned place preference induced by cocaine and morphine (experiment 4). Histograms represent the time (mean + SEM) spent during the 20 min test session on the floor texture previously paired with cocaine or morphine. The *horizontal dashed line* indicates the chance level (no preference). Cocaine (2 mg/kg SC) or morphine (4 mg/kg SC) were administered immediately before the four "even" conditioning sessions. SR 141716 or its vehicle was injected IP 30 min before cocaine or morphine. Animals were drug-free during the test session (the number of rats per group is indicated in the histograms). *$P < 0.05$, **$P < 0.01$ versus associated group given cocaine or morphine alone (Dunnett's t-test after ANOVA)

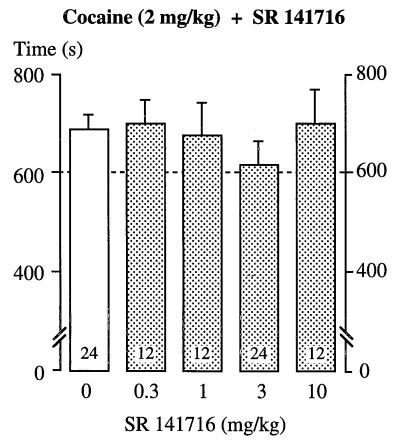

Fig. 5 Effects of SR 141716 on the establishment of cocaine-induced conditioned place preference (experiment 5). Histograms represent the time (mean + SEM) spent during the 20-min test session on the floor texture previously paired with cocaine. The *horizontal dashed line* indicates the chance level (no preference). Cocaine (2 mg/kg SC) was injected immediately before each of the four "even" conditioning sessions. SR 141716 or its vehicle was administered IP 30 min before the test session (the number of rats per group is indicated in the histograms). Statistical analysis failed to reveal differences for these values

due to a significant reduction of locomotion in groups given 1, 3 and 10 mg/kg of SR 141716 (number of quadrants crossed: controls: 77 ± 6; SR 0.3 mg/kg: 65 ± 7; SR 1 mg/kg: 47 ± 8; t = 3.48; $P < 0.01$; SR 3 mg/kg: 54 ± 6; t = 3.20; $P < 0.01$; SR 10 mg/kg: 39 ± 7; t = 4.34; $P < 0.01$).

Experiment 6: effects of SR 141716 on the cocaine-induced stimulation of motor activity

SR 141716, administered alone, did not affect rats motor activity during both the 30-min and 60-min observation periods [both F(3,45) < 1; NS]. Animals given cocaine (4 mg/kg) crossed more light beams than vehicle-injected rats, whatever the time-interval considered (one-tailed Student's t = 1.87; $P < 0.05$ and t = 2.39; $P < 0.02$). This cocaine-induced hyperactivity was not significantly modified by SR 141716 over either 30 or 60 min [F(3,27) = 0.36 and = 1.59, respectively; NS] (Table 1).

Discussion

The present study provides clear evidence that preconditioning administration of the CB_1 receptor antagonist, SR 141716, impaired the acquisition of CPP induced by classical reinforcers such as cocaine,

morphine and food. This was observed in the range of doses active to counteract the characteristic in vivo effects of cannabinoid agonists (see references in the Introduction section), indicating the involvement of CB_1-related processes in the perception of the rewarding value of reinforcers, even when these reinforcers did not directly interact with cannabinoid systems. The fact that the reversal by SR 141716 of the CPP supported by each appetitive agent did not exactly follow the same dose-effect relationship can indicate that the involvement of cannabinoid systems would differ according to the exact mechanism which subserve the action of each reinforcer. As distinct from the recent finding that SR 141716 established CPP (Sañudo-Peña et al. 1997), no place conditioning was found, in two independent experiments, with SR 141716, suggesting that an endogenous cannabinoid tone does not exist under the present experimental conditions. The reason for such contrasting results is not immediately clear.

Several points deserve discussion before a more detailed analysis of the possible role of cannabinoid systems in reward processes. First, whereas SR 141716 reversed all the pharmacological and behavioural effects of CB receptor agonists, it was relatively ineffective in counteracting morphine-induced analgesia (Compton et al. 1996; Lichtman and Martin 1997), and failed to reverse the stimulation of locomotor activity produced by cocaine (present study). Such an absence of antagonism could be accounted for by some pharmacokinetic effects due to differences in the route of administration (morphine analgesia), the dose used, the period of observation and/or the level of food deprivation (cocaine hyperactivity). However, it cannot be excluded that appetitive and non-appetitive actions of these two drugs could be subserved, at least in part, by different neurobiological substrates, an activation of CB_1 receptors being specifically involved in reward-related processes.

Second, cocaine and several other rewarding drugs tend to stimulate motor activity, an effect which may become conditioned and contribute to the establishment of incentive learning (Carr et al. 1989). Locomotion was not recorded during the conditioning sessions, but a single injection of SR 141716 prior to the test session (experiment 5) reduced the number of transitions between quadrants of the open field. Thus, the antagonism by SR 141716 of the CPP supported by cocaine would be accounted for by a blockade of motor stimulation during the conditioning sessions. This seems unlikely, since transitions between quadrants during the test session were not affected by pre-conditioning administrations of cocaine alone (no conditioned hyperactivity), or of cocaine plus SR 141716. In addition, when measured in actimeters, neither the spontaneous activity nor the hyperactivity induced by acute cocaine, was affected by SR 141716. Taken together, these results indicate that the reduction of cocaine-induced CPP is unlikely to be accounted for by secondary, non-specific effects of SR 141716 on motor activity.

Third, incentive learning has been suggested to depend on both the affective and the memory-enhancing effects of the reinforcer tested (see Carr et al. 1989). Conversely, impaired associative processes could result in the failure of reinforcers to establish place conditioning. There is no evidence for amnestic-like effects of SR 141716. On the contrary, this drug has been reported to enhance arousal (Santucci et al. 1996) and facilitate short-term memory in a rodent test of social recognition (Terranova et al. 1996). Therefore, the reduction by SR 141716 of cocaine-, morphine-, and food-induced CPP is very probably not accounted for by impaired associative processes.

Fourth, SR 141716 produced a dose-dependent reduction of food intake which would participate to the alteration of CPP. In particular, SR 141716 impaired the progressive increase in sucrose consumption observed in control rats during the successive conditioning sessions, while the initial reduction of usual food intake vanished upon repeated daily injections. Therefore, it cannot be excluded that a conditioned taste aversion due to the association of sweet taste to some effects of SR 141716 could account for the antagonism of food-induced CPP. However, this is unlikely since SR 141716 alone seems not to induce "unpleasant" effects, at least as assessed in the present procedure. In addition, a selective reduction of sucrose intake by single administrations of SR 141716 was reported in various food consumption paradigms, even in animals familiarised to sucrose and testing conditions (Arnone et al. 1997). Taken together, these results suggest that SR-141716 might lessen the incentive value of the ingested food.

Therefore, the present study indicates that SR 141716 could specifically counteract reward-related behaviours, whatever the specific factors involved in the action of each reinforcer, and that cannabinoid (CB_1) receptors could be crucially involved in the neurobiological events evoked by appetitive reinforcers. However, this does not necessarily mean that a permanent endogenous cannabinoid tone exists to ensure the organism a basal hedonic level. Indeed, on its own, SR 141716 did not support CPA, as it should be observed under this assumption. Thus, it can be postulated that cannabinoid-related processes are elicited and maintained by pleasant reinforcements.

SR 141716 antagonised the *acquisition* but not the *expression* of cocaine-induced CPP. Although, in a first experiment, a 20% reduction of the time spent on the texture previously paired to cocaine was observed in rats given SR-141716 (3 mg/kg) before the test session, this effect failed to reach a statistically significant level and was neither reproduced nor observed with a larger dose in a second experiment. This suggests that the processes triggered by unconditioned and conditioned rewards differ and that only the acquisition stage

requires a cannabinoid link. Similar dissociations have been reported with DA receptor antagonists, and numerous studies indicated that different neuronal mechanisms are involved in the two conditioning stages (see Beninger and Herz 1986; Cervo and Samanin 1995). In fact, such differences seem a general feature of place conditioning studies, and might simply result from the fact that the antagonists are administered repeatedly during the conditioning stage, whereas they are usually given only once, before a single test session. Therefore, it cannot be excluded that, on sub-chronic treatment, SR 141716 would have also counteracted the expression of cocaine-induced CPP.

As pointed out in the Introduction section, there is no clear evidence that cannabinoid agonists exhibit hedonic-like properties in animals. In the present study, WIN 55212-2 induced CPA, as already reported for other CB receptor agonists (Lepore et al. 1995; Parker and Gillies 1995; McGregor et al. 1996; Sañudo-Peña et al. 1997), and this aversive effect was antagonised by SR 141716, providing good evidence for the involvement of CB_1 receptors. Although biphasic effects have been described with cannabinoid agonists – for instance, motor stimulation at low doses and profound hypolocomotion and catalepsy at larger doses (Gough and Olley 1977; Sakurai et al. 1985; Souilhac et al. 1995; McGregor et al. 1996) – this seems not the case for the incentive effects, since WIN 55212-2 did not induce CPP, even at doses 100-fold lower than those supporting CPA. It cannot be excluded that, perhaps due to its slight preferential affinity for the CB_2 vs. the CB_1 receptor subtype (as determined at cloned human receptors) (Showalter et al. 1996), an appetitive potential of WIN 55212-2 would be masked by some adverse effects. Indeed, hypothermia and/or nausea due to cannabimimetics might result in a general feeling of malaise in rats (Parker and Gillies 1995; McGregor et al. 1996) as sometimes reported in humans with large doses of Δ^9-THC (Hollister 1986). Thus, on their own, CB receptor agonists did not elicit the processes necessary to induce a pleasurable state, at least as assessed in animals by place conditioning procedures.

In summary, the present study, using SR 141716 as a tool, clearly shows that the blockade of central CB_1 receptors impairs the establishment of CPP, and their stimulation by WIN 55212-2 induces CPA. This suggests that the activation of reward systems could be under the permissive control of some complex, CB_1-related cannabinoid processes which are required for the perception of the incentive value of positive reinforcements, whatever their nature. It can be postulated that the activation of such a cannabinoid system by an endogenous ligand may play an essential role in eliciting and then in maintaining appetitive behaviour. It remains to be established whether the present results can be extended to other appetitive behaviours, and more specifically, whether the blockade of CB_1 receptors may also interact with self-administration or intracranial self-stimulation. In addition, it could be interesting to investigate if cannabinoid systems are also involved in the incentive processes associated to negative, non-cannabinoid, reinforcements and thereby modulate the perception of the incentive value of reinforcers, whether they are rewarding or aversive.

References

Arnone M, Maruani J, Chaperon F, Thiébot MH, Poncelet M, Soubrié P, Le Fur G (1997) Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptor. Psychopharmacology 132:104–106

Beninger R, Herz R (1986) Pimozide blocks establishment but not expression of cocaine-induced environment-specific conditioning. Life Sci 38:1425–1431

Bozarth M (1991) The mesolimbic dopamine system as a model of reward system. In: Willner P, Scheel-Krüger J (eds) The mesolimbic dopamine system: from motivation to action. Wiley, Chichester, pp 301–330

Carr GD, Fibiger HG, Phillips AG (1989) Conditioned place preference as a measure of drug reward. In: Liebman JM, Cooper SJ (eds) The neuropharmacological basis of reward. Clarendon Press, Oxford, pp 264–319

Castañeda E, Moss DE, Oddie SD, Whishaw IQ (1991) THC does not affect striatal dopamine release: microdialysis in freely moving rats. Pharmacol Biochem Behav 40:587–591

Cervo L, Samanin R (1995) Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. Brain Res 673:242–250

Chaperon F, Thiébot MH (1996) Effects of dopaminergic D_3-receptor preferring ligands on the acquisition of place conditioning in rats. Behav Pharmacol 7:105–109

Chen J, Paredes W, Li J, Smith D, Lowinson J, Gardner E (1990a) Δ^9-Tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology 102:156–162

Chen J, Paredes W, Lowinson J, Gardner E (1990b) Δ^9-Tetrahydrocannabinol enhances presynaptic dopamine efflux in medial prefrontal cortex. Eur J Pharmacol 190:259–262

Compton D, Aceto M, Lowe J, Martin B (1996) In vivo characterization of a specific cannabinoid receptor antagonist (SR 141716): inhibition of Δ^9-tetrahydrocannabinol-induced responses and apparent agonist activity. J Pharmacol Exp Ther 277:586–594

Corcoran M, Amit Z (1974) Reluctance of rats to drink hashish suspensions: free-choice and forced consumption, and the effects of hypothalamic stimulation. Psychopharmacologia 35: 129–147

Gardner E, Paredes W, Smith D, Donner A, Milling C, Cohen D, Morrison D (1988) Facilitation of brain stimulation reward by delta-9 tetrahydrocannabinol. Psychopharmacology 96:142–144

Gough AL, Olley JE (1977) Δ^9-Tetrahydrocannabinol and the extrapyramidal system. Psychopharmacology 54: 87–99

Guyon A, Assouly-Besse F, Biala G, Puech AJ, Thiébot MH (1993) Potentiation by low doses of selected neuroleptics of food-induced conditioned place preference in rats. Psychopharmacology 110:460–466

Harris RT, Waters W, McLendon D (1974) Evaluation of reinforcing capability of delta-9-tetrahydrocannabinol in monkeys. Psychopharmacology 37:23–29

Hollister L (1986) Health aspects of cannabis. Pharmacol Rev 38: 1–19

Howlett A (1995) Pharmacology of cannabinoid receptors. Annu Rev Pharmacol Toxicol 35:607–634

Leite JR, Carlini EA (1974) Failure to obtain "cannabis-directed behavior" and abstinence syndrome in rats chronically treated with *Cannabis sativa* extracts. Psychopharmacologia 35:133–145

Lepore M, Vorel SR, Lowinson J, Gardner E (1995) Conditioned place preference induced by Δ^9-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. Life Sci 56:2073–2080

Lichtman AH, Martin BR (1996) Δ^9-Tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharmacology 126:125–131

Lichtman AH, Martin BR (1997) The selective cannabinoid antagonist SR 141716A blocks cannabinoid-induced antinociception in rats. Pharmacol Biochem Behav 57:7–12

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564

McGregor IS, Issakidis CN, Prior G (1996) Aversive effects of the synthetic cannabinoid CP 55,940 in rats. Pharmacol Biochem Behav 53:657–664

Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365:61–65

Onaivi ES, Green MR, Martin BR (1990) Pharmacological characterization of cannabinoids in the elevated plus maze. J Pharmacol Exp Ther 253:1002–1009

Parker LA, Gillies T (1995) THC-induced place and taste aversion in Lewis and Sprague-Dawley rats. Behav Neurosci 109:71–78

Pério A, Rinaldi-Carmona M, Maruani J, Barth F, Le Fur G, Soubrié P (1996) Central mediation of the cannabinoid cue: activity of a selective CB1 antagonist, SR 141716A. Behav Pharmacol 7:65–71

Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Néliat G, Caput D, Ferrara P, Soubrié P, Brelière JC, Le Fur G (1994) SR 141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350:240–244

Rinaldi-Carmona M, Barth F, Héaulme M, Alonso R, Shire D, Congy C, Soubrié P, Brelière JC, Le Fur G (1995) Biochemical and pharmacological characterisation of SR 141716A the first potent and selective brain cannabinoid receptor antagonist. Life Sci 56:1941–1947

Sakurai Y, Ohta H, Shimazoe T, Kataoka Y, Fujiwara M, Ueki S (1985) Δ^9-Tetrahydrocannabinol elicited ipsilateral circling behavior in rats with unilateral nigral lesion. Life Sci 37:2181–2185

Santucci V, Storme JJ, Soubrié P, Le Fur G (1996) Arousal-enhancing properties of the CB1 cannabinoid receptor antagonist SR 141716 in rats as assessed by electroencephalographic spectral and sleep-waking cycle analysis. Life Sci 58:103–110

Sañudo-Peña MC, Tsou K, Delay ER, Hohman AG, Force M, Walker JM (1997) Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. Neurosci Lett 223: 125–128

Showalter VM, Compton DR, Martin BR, Abood ME (1996) Evaluation of binding in transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. J Pharmacol Exp Ther 278:989–998

Souilhac J, Poncelet M, Rinaldi-Carmona M, Le Fur G, Soubrié P (1995) Intrastriatal injection of cannabinoid receptor agonists induced turning behavior in mice. Pharmacol Biochem Behav 51:3–7

Takahashi RN, Singer G (1979) Self-administration of delta 9-tetrahydrocannabinol by rats. Pharmacol Biochem Behav 11:737–740

Terranova J-P, Storme J-J, Lafon N, Pério A, Rinaldi-Carmona M, Le Fur G, Soubrié P (1996) Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716. Psychopharmacology 126:165–172

Wiley JL, Barrett RL, Lowe J, Balster RL, Martin BR (1995a) Discriminative stimulus effects of CP 55,940 and structurally dissimilar cannabinoids in rats. Neuropharmacology 34: 669–676

Wiley JL, Lowe JL, Balster RL, Martin BR (1995b) Antagonism of the discriminative stimulus effects of delta 9-tetrahydrocannabinol in rats and rhesus monkeys. J Pharmacol Exp Ther 275:1–6