Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 483 (2004) 55–63
www.elsevier.com/locate/ejphar

Behavioral mechanisms underlying inhibition of food-maintained responding by the cannabinoid receptor antagonist/inverse agonist SR141716A

Jean De Vry*, Rudy Schreiber, Gisela Eckel, Klaus Rüdiger Jentzsch

CNS Research, Bayer HealthCare, Aprather Weg 18a, 42096 Wuppertal, Germany

Received 5 June 2003; received in revised form 12 September 2003; accepted 10 October 2003

Abstract

This study investigated the possible behavioral mechanisms underlying the anorectic effect of the cannabinoid CB_1 receptor antagonist/inverse agonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A). Male or female rats were food-restricted and trained to emit stable responding in daily 10-min, fixed ratio 10 food-reinforced operant sessions. Under these conditions, as well as under free-feeding conditions, SR141716A inhibited food-maintained responding (ED_{50} values ranging from 0.92 to 2.52 mg/kg, i.p.). In the same operant procedure, SR141716A suppressed intracranial self-stimulation with a potency which was slightly lower than the anorectic potency (ED_{50}: 4.50 mg/kg). As assessed during a 10-min test period SR141716A (1–10 mg/kg) did not affect activity counts; suggesting that the observed inhibition of operant behavior is not a direct consequence of impairment of locomotor activity. SR141716A, however, attenuated saccharin-preference in a conditioned taste aversion paradigm (ED_{50}: 6.45 mg/kg). Although the data support the suggestion that the anorectic effect of SR141716A results from an attenuating effect on the rewarding effect of food, the contribution of drug-induced aversion/malaise cannot be excluded.

Keywords: Appetite; Cannabinoid CB_1 receptor; Conditioned taste aversion; Ingestive behavior; Intracranial self-stimulation; Locomotor activity; Operant behavior; (Rat)

1. Introduction

The selective, centrally active, cannabinoid CB_1 receptor antagonist/inverse agonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A; Rinaldi-Carmona et al., 1994) has been reported to inhibit food intake in a variety of species and models of ingestive behavior (e.g., Arnone et al., 1997; Colombo et al., 1998a; Freedland et al., 2000; Kirkham and Williams, 2001a; Rowland et al., 2001; Simiand et al., 1998). The crucial role of cannabinoid CB_1 receptors in the anorectic effect of SR141716A is underscored by the finding that it is abolished in transgenic mice lacking cannabinoid CB_1 receptors (Di Marzo et al., 2001). This finding and the fact that cannabinoids are well known to increase food intake indicates that the endogenous cannabinoid (endocannabinoid) system plays a role in the control of ingestive behavior (Kirkham and Williams, 2001b).

As the endocannabinoid system is thought to be involved in the brain reward system, it has been argued that the anorectic effect of SR141716A results from an attenuating effect on feeding-related reward processes (for discussion, see Kirkham and Williams, 2001b). It remains unclear, however, whether the latter effect is due to (1) an attenuating effect of the compound on the orosensory characteristics of food (“palatability hypothesis”), or to (2) an attenuation of the appetitive or incentive aspects of feeding behavior (“appetite hypothesis”; for discussion, see Higgs et al., 2003; Kirkham and Williams, 2001b). In support of the palatability hypothesis, initial studies (e.g., Arnone et al., 1997; Simiand et al., 1998) reported that SR141716A preferentially affects the intake of highly palatable ingesta (food and liquids with a sweet taste). However, the finding that the intake of a highly palatable sucrose solution is reduced by SR141716A in intact rats, but not in rats with a gastric cannula through which ingested sucrose solutions are

* Corresponding author. Tel.: +49-202-365105; fax: +49-202-365156.
E-mail address: jean.vry.jv1@bayer-ag.de (J. De Vry).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.10.012

immediately recovered (sham-feeding), suggests that other mechanisms may underlie the anorectic effect of the compound (Kirkham and Williams, 2001b). Because the intake of bland laboratory chow was reduced by SR141716A after food-restriction, but not under free-feeding conditions, it can be argued that the anorectic effect of SR141716A results from an inhibition of appetitive processes (Kirkham and Williams, 2001b). In addition, the finding that the intake of sucrose pellets in an operant paradigm (Pério et al., 2001) is not more potently suppressed by SR141716A than the intake of normal pellets (Freedland et al., 2000) suggests that the anorectic effect of the compound does not depend on the palatability of the ingesta.

Although the latter studies may imply an effect of SR141716A on appetitive processes, it cannot be ruled out that it is just a consequence of motor/performance deficits. With respect to the latter issue, it has been reported that the compound fails to affect locomotor activity, but these studies have used locomotor assessment times which did not match the time periods used to assess the anorectic effect [e.g., in the Freedland et al. (2000) study the operant session lasted 30 min, whereas the locomotor activity study lasted for 4 h]. Interestingly, in an operant study in which beer-reinforced behavior and locomotor activity was simultaneously measured, it was found that SR141716A suppressed intake in the same dose range which inhibited general behavior (Gallate and McGregor, 1999). Finally, as it has been reported that SR141716A induces emesis in the least shrew (Darmani, 2001) and potentiates lithium-induced conditioned rejection reactions in rats (Parker et al., 2003), it cannot be excluded that drug-induced aversive effects are involved in the anorectic effect of the compound.

The present study was performed to test further possible behavioral mechanisms underlying the anorectic effect of SR141716A. The first experiment tested whether feeding status affects the anorectic effect of the compound, as assessed in a 10-min, fixed ratio 10 operant food-reinforced paradigm. Because of its limited duration, the procedure is highly sensitive to appetitive processes (for discussion, see De Vry et al., in press). In order to manipulate the appetitive component of the model, the effect of SR141716A on food-maintained responding was tested in rats which were either food-restricted or which were given free access to food before the test session. It was argued that if food-restriction resulted in a more potent anorectic effect of the compound, a suppressive effect on appetite could be inferred.

In a subsequent experiment, the efficacy of SR141716A to suppress intracranial self-stimulation was assessed in the same 10-min, fixed ratio 10 operant paradigm. Special attention was given to obtain a similar rate of responding (by adjusting the stimulation parameters) as obtained in the food-reinforced operant procedure. Because the latter experiment was performed in female rats, the same animals were subsequently retrained under the same experimental conditions to lever-press for food, and tested again with SR141716A. This experiment was performed to test whether SR141716A differentially affects operant behavior maintained by food reinforcement and by direct stimulation of the brain reward system. It was argued that absence of a difference in suppressive potency would be supportive for the suggestion that the anorectic effect of SR141716A results from an attenuating effect on feeding-related reward processes.

To test whether the suppressive effect of SR141716A on operant behavior might be confounded by an effect on general activity, the effect of the compound on locomotor activity was assessed during a 10-min session in rats habituated to the test environment. A last experiment tested the possibility that the anorectic effect is a consequence of drug-induced aversive effects. To this end, it was investigated whether SR141716A attenuates saccharin-preference in a conditioned taste aversion paradigm.

2. Material and methods

2.1. Animals

Female (intracranial self-stimulation followed by operant food intake study) and male (all other studies) Wistar rats were purchased from Harlan-Winkelmann (Hsd/cpb: WU, Borchen, Germany). Body weight upon arrival at the laboratory was around 160 (females) and 200 g (males), which gradually increased during the course of the studies to 210–250 and 350–530 g, respectively. Rats were individually housed in Macrolon® type 3 cages (22 × 37 cm, height 15 cm) under a normal 12-h light period (lights on at 7:00 a.m.). Room temperature was maintained at 20–22 °C. Except for the operant food intake and conditioned taste aversion studies, tap water and food (standard pellets; Ssniff Spezialdiäten, Soest, Germany) was supplied ad libitum in the home cages. In the case of the operant food intake studies, tap water was freely available, but the animals were food-restricted in that food access was limited to daily portions of about 13 g (resulting in body weights which were about 80% of free-feeding body weight). In the case of the conditioned taste aversion study, food was freely available but availability of tap water was restricted according to the schedule described further. The procedures followed the guidelines for the use of animals, as given by the German government, and were approved by the local authorities (Regierungspräsidium Düsseldorf, Germany).

2.2. Experimental procedures and design

2.2.1. Operant food intake experiment in male rats

Operant food intake sessions were performed in sound- and light-attenuated standard operant chambers (modular test cage system, model EW-10 SF, Coulborn Instruments, Lehigh Valley, PA, USA). The chambers were equipped with two levers equidistant from a food tray between the levers. Food reinforcement (45 mg precision pellets; Bio-

Serv, Frenchtown, NJ) was delivered by an automated food dispenser located outside of the chamber. Data collection and experimental contingencies were programmed using OPN software (developed by D.G. Spencer, M.W. Emmett-Oglesby and D. Arnoult) on a PC interfaced with the operant chambers. A white houselight was switched on during the sessions, which were conducted during the light phase of the day/night cycle, between 9:00 a.m. and 3:00 p.m.

After initial shaping to press on the left lever for food reinforcement, male food-restricted rats ($n = 32$) were trained to achieve a stable baseline of operant behavior on a fixed ratio 10 schedule of reinforcement during daily 10-min sessions (five sessions per week, Monday through Friday). After reaching stable baselines of operant responding (about 500–900 responses/session), the effect of SR141716A (0, 1, 3 and 10 mg/kg, i.p., $t - 30$ min) on food-maintained responding was investigated under the same food-restriction conditions as used during the training phase of the experiment (i.e., food-restricted rats). In a second experiment, the animals had unlimited access to food between the regular training session (on Tuesday) and the next daily 10-min test session (Wednesday), during which the effect of SR141716A (0, 1, 3 and 10 mg/kg, i.p., $t - 30$ min) on food-maintained responding was again assessed. For each test with SR141716A (food-restriction, or no food-restriction), four groups of rats ($n = 7–8$/group) were tested (generally on Wednesday) with either vehicle, or one of three doses of the compound. SR141716A was tested twice under each feeding condition (data were pooled for statistical analysis).

2.2.2. Intracranial self-stimulation and operant food-intake experiment in female rats

The intracranial self-stimulation paradigm was partially based on the method described by Kling-Petersen and Svensson (1993). For this experiment, female rats were used as their growth rate is much slower than that of males; thereby maximizing the chance that electrode placement remained stable over longer periods of time). Rats ($n = 12$) were implanted with a twisted bipolar electrode (Plastic One, no. 303/1, length cut at 9.0 and 9.7 mm) aimed at the median forebrain bundle at the level of the lateral hypothalamus. Surgical anesthesia was induced by a mixture of ketamine hydrochloride (Ketavet®, Pharmacia-Upjohn, Vienna, Austria, 72 mg/kg, i.p.) and xylazine hydrochloride (Rompun® 2%, Bayer, Leverkusen, Germany, 9.6 mg/kg, i.p.). With the skull held horizontal between bregma and lambda, the stereotaxic coordinates were -3.0 (frontal), $+1.5$ (lateral) and -8.7 (horizontal), according to König and Klippel (1963). Three stainless steel screws were fixed to the skull prior to placement of the electrode, and screws and electrode were then fixed together using dental cement (Palavit®, 55 VS, Kulzer, Wehrheim, Germany). After surgery, the animals were treated with an antibiotic (Borgal® 7.5%, Hoechst Roussel Vet, Unterschleissheim, Germany, 0.1 ml/rat, s.c.) and they were allowed at least one week recovery before training began.

Experiments were performed in the same operant chambers as used in the food-reinforced operant procedure. After placing the rat in the test cage, a lead connected to a commutator was screwed to the electrode. The commutator (Plastic One, no. SL2C) allowed the animal to move freely around the cage. A lead from the commutator was connected to the stimulator (no. E13-51, Coulbourn Instruments) and the applied stimulation was monitored using a standard laboratory oscilloscope.

The rats were trained to achieve a stable baseline of operant behavior on a fixed ratio 10 schedule of reinforcement during daily 10-min sessions (five sessions per week, Monday through Friday). The stimulation following each 10th lever press consisted of a 0.3-s train of biphasic rectangular pulses of 8.332-ms duration [duration of pulse 1 (positive), interpulse, and pulse 2 (negative): 2.083 ms each]; resulting in a frequency of 120 Hz. In order to obtain a response rate approaching that of the food-reinforced procedure (i.e., 500–900 responses/10-min session), the current intensity was individually set for each rat (range: 43–85 μA). After reaching stable baseline responding, SR141716A (0, 1, 3 and 10 mg/kg, i.p) was administered 30 min before the 10-min test session (each dose accompanied by a test with vehicle). In the case of 3 mg/kg SR141716A, a replication was performed (both with drug and vehicle) and the data were pooled for statistical analysis. Rats were repeatedly tested; receiving, in general, one dose per week. A compound was only tested if response rate had returned to the level of the previously obtained baseline.

After termination of the dose–response study with SR141716A in the intracranial self-stimulation paradigm, the remaining three rats were food-restricted and trained to achieve stable operant responding in the same 10-min, fixed ratio 10, food-reinforced procedure as described above. After reaching stable baselines of operant responding (about 200–400 lever responses/session), each rat was tested twice per week, and was injected twice with vehicle and twice with 1, 3 and 10 mg/kg SR141716A (i.p., $t - 30$ min), given in a randomized order.

2.2.3. Locomotor activity experiment

The locomotor activity study was performed with three identical Activity and Motility Devices (Technical and Scientific Equipment, Bad Homberg, Germany), placed in a sound-insulated cubicle. Male rats were placed individually in Macrolon® type 3 cages (fresh cages for each animal), which were located on the surface (35×34.5 cm) of the apparatus, and activity counts were registered by means of distortions of a magnetic field induced by any movement of the animal. Subjects were habituated to the test environment during two consecutive daily 10-min sessions, the second session being preceded by an i.p. injection with vehicle ($t - 30$ min). On the test day, the groups ($n = 6$/group) were treated with SR141716A (0, 1, 3

and 10 mg/kg, i.p., $t-30$ min), and activity counts were performed during a 10-min period. Sessions were performed between 9:00 a.m. and 3:00 p.m.

2.2.4. Conditioned taste aversion experiment

In general, the method described by de Beun et al. (1996) was followed. Thus, 24 h before the first session, male rats were water deprived and fluid access was from then on restricted to daily experimental sessions of 15 min, which took place individually in a Macrolon® type 3 test cage. After each session, the animals returned to their respective home cages. Food was freely available in the home cages throughout the procedure, but was not available during the sessions. For a given subject, all six sessions required to complete a conditioned taste aversion took place in the same test cage and the cages were not cleaned between sessions. Animals designated to the same experimental group ($n=8$ per group) were run in parallel. During the first four sessions, both bottles contained plain tap water. This phase of the procedure gave the animals the opportunity to learn to drink a reasonable amount of water in a short period of time and to establish a relatively stable baseline value of water intake. For the fifth session (conditioning session), both bottles were filled with a novel fluid (i.e., a 0.1% W/V saccharin solution) and immediately after completion of this session the animals were injected with either the appropriate vehicle or the test drug. SR141716A was tested at the following doses: 0, 1, 3 and 10 mg/kg, i.p. Between the conditioning session and the final test session for conditioned taste aversion, the animals were left undisturbed for about 72 h (wash-out period) and during the first 48 h of this period they had free access to tap water in their home cages until they were again deprived of water. During this last session, one bottle contained the saccharin solution used for conditioning and the other bottle was filled with tap water. To control for location bias, the saccharin solution was presented in the left bottle for half of the animals in each group and in the right bottle for the other half. By measuring the amount of fluid consumed from both bottles separately, drug-induced conditioned taste aversion could be determined by comparison of the relative saccharin intake in the drug-treated groups and their vehicle-treated controls.

2.3. Drugs

SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride] was synthesized by the Chemistry Department of Bayer (Wuppertal, Germany) and was dissolved in 2.5–5% Solutol® HS 15 (12-hydroxystearic acid ethoxylate, BASF, Ludwigshafen, Germany), 2.5–5% ethanol (ethanol absolute, 99.8%, Riedel-de Haën, Seelze, Germany) and distilled water or 0.9% NaCl (saline). Application volume was 2 ml/kg body weight (operant behavior and locomotor activity assay) or 5 ml/kg (conditioned taste aversion assay).

2.4. Data analysis

For all operant experiments (food- or brain stimulation-maintained), the number of responses obtained during a test session (after administration of SR141716A or vehicle) was analyzed by analysis of variance (ANOVA), followed by a post hoc Tukey *t*-test. Individual locomotor activity counts were accumulated over the 10-min test period and analyzed by ANOVA. For the conditioned taste aversion paradigm, individual saccharin preference values were calculated as the ratio of saccharin consumption divided by total fluid consumption, as obtained on the test session, and analyzed by ANOVA; followed by a post hoc Tukey *t*-test. For calculation of the effective dose$_{50}$ (ED$_{50}$) values and their 95% confidence limits, data obtained with each dose of a particular test drug were expressed as percentage reduction of responding, activity counts or saccharin preference, as compared with vehicle treatment, and these percentages were transformed by log-probit analysis before submission to least-square analysis. ED$_{50}$ values with nonoverlapping 95% confidence limits were considered to be statistically significant. The lowest dose which resulted in a statistically significant ($P<0.05$) effect, as compared with vehicle treatment was considered to be the minimal effective dose (MED).

3. Results

3.1. Operant food intake experiments in male rats

During the period of pharmacological testing, the food-restricted rats showed a stable and relatively high level of operant responding (500–900 responses/10-min session, obtained after 40 training sessions, and resulting in a mean intake of 2.3–4.1 g food pellets). SR141716A induced a dose-dependent reduction in the number of responses [$F(3,56)=56.12$, $P<0.001$], with a MED of 1 mg/kg (Fig. 1A) and an ED$_{50}$ value (95% confidence limits) of 2.52 (1.40–4.54) mg/kg. At the highest dose tested (10 mg/kg), the suppression of food-maintained responding was almost complete (89% inhibition of number of responses, as compared with vehicle treatment). As expected, giving the rats free access to food during the 24-h period between the baseline session and the test session resulted in a strong decrease in operant responding (52% decrease in number of responses, as compared with baseline session, $P<0.001$; Fig. 1B, left bar representing vehicle-treated control group). However, also after free-feeding, SR141716A induced a potent and dose-dependent reduction in the number of responses [$F(3,54)=12.04$, $P<0.001$; Fig. 1B]; with a MED of 1 mg/kg and an ED$_{50}$ value (95% confidence limits) of 0.92 (0.40–2.13) mg/kg. Food-maintained responding was completely suppressed after 10 mg/kg SR141716A (100% inhibition of number of responses, as compared with vehicle treatment).

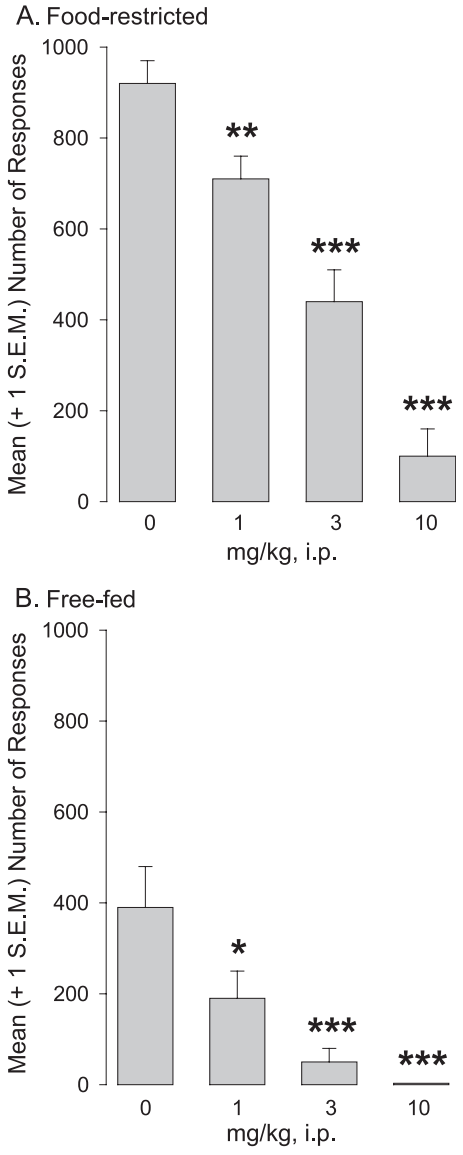

Fig. 1. Effect of SR141716A (i.p., $t-30$ min) on operant food intake in (A) food-restricted and (B) free-fed male rats. Rats were trained to respond on a fixed ratio 10 schedule of food reinforcement during daily 10-min sessions. On the nontreated baseline session preceding the test session, the mean number (+1 S.E.M.) of responses in the different groups ranged from 836 (40) to 890 (40). $n=14-15$ per treatment. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared with vehicle treatment.

3.2. Intracranial self-stimulation and operant food intake experiment in female rats

Four out of 12 rats trained to self-administer electrical brain stimulation in a 10-min, fixed ratio 10 operant paradigm attained a stable and relatively high level of responding (700–750 responses/session, obtained after 45 training sessions). During the period of pharmacological testing, these rats (one rat was discarded as it had lost its electrode) showed a level of operant responding which was almost comparable in magnitude as the level obtained in the food-restricted male rats in the food-maintained operant paradigm. SR141716A induced a dose-dependent reduction in the number of responses [$F(3,20)=13.91$, $P<0.001$]; with a MED of 3 mg/kg (Fig. 2A) and an ED_{50} value (95% confidence limits) of 4.50 (0.96–20.97) mg/kg. At the highest dose tested, SR141716A suppressed electrical brain stimulation-maintained responding by 65% (as compared with vehicle treatment). After termination of the intracranial self-stimulation experiment, the three remaining rats

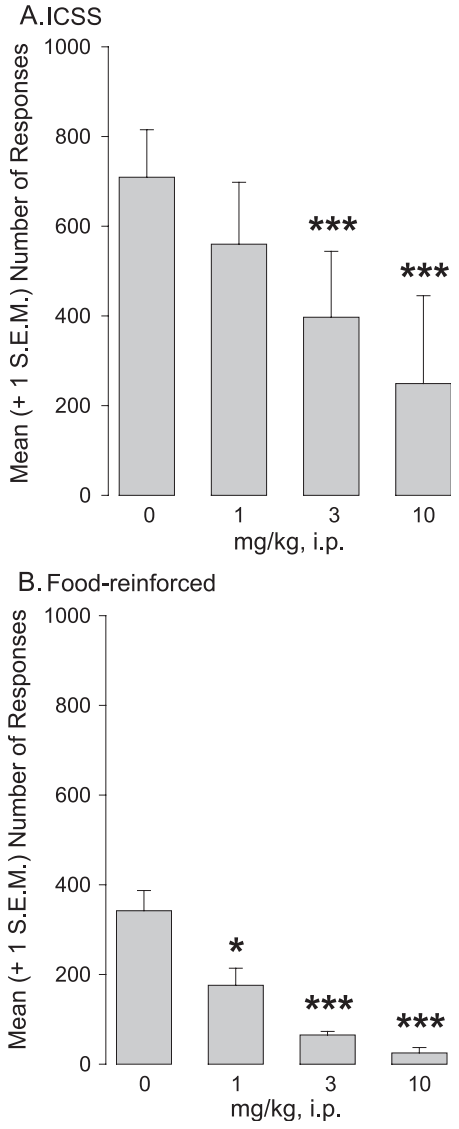

Fig. 2. Effect of SR141716A (i.p., $t-30$ min) on (A) operant intracranial self-stimulation (ICSS) and (B) operant food intake in female rats. Rats were first trained to respond for electrical brain stimulation in the medial forebrain bundle on a fixed ratio 10 schedule of reinforcement, and tested with SR141716A (10-min session). Thereafter, the same rats were food-restricted and trained to respond for food on the same reinforcement schedule, and tested again with SR141716A (10-min session). On the nontreated baseline session preceding the test session, the mean number (+1 S.E.M.) of responses ranged from 700 (196) to 735 (184) in the ICSS paradigm, and from 326 (32) to 389 (52) in the food-reinforced paradigm. $n=3-12$ (A) and $n=6$ (B) per treatment. $^{*}P<0.05$, $^{***}P<0.001$ as compared with vehicle treatment.

reached stable responding in the operant food-reinforced procedure after 34 training sessions. Their baseline response rate was clearly lower (about 200–400 responses/10-min session) than that of their food-restricted male counterparts. SR141716A induced a potent and dose-dependent reduction in number of responses [$F(3,20)=22.21$, $P<0.001$]; with a MED of 1 mg/kg (Fig. 2B) and an ED_{50} value (95% confidence limits) of 0.97 (0.29–3.24) mg/kg. At the highest dose tested, the suppression of food-maintained responding was almost complete (i.e., 93% inhibition of number of responses, as compared with vehicle treatment). SR141716A appeared to suppress food-reinforced responding in a slightly more potent manner as compared with its suppression of brain stimulation-reinforced responding (ED_{50} values were, however, not significantly different).

3.3. Locomotor activity experiment

On the habituation session preceding the test session, mean activity counts (+1 S.E.M.) of the four groups ranged from 688 (128) to 747 (98) during the 10-min session, and they were not statistically different from each other [$F(3,20)=1.97$; $P>0.05$]. Although there was no significant effect of SR141716A on activity counts [$F(3,20)=0.96$; $P>0.05$; Fig. 3], post hoc analysis indicated a weak trend for a reduction at 10 mg/kg (33% reduction as compared with vehicle treatment, $P=0.13$).

3.4. Conditioned taste aversion experiment

SR141716A induced a reduction in % saccharin preference [$F(3,28)=5.73$, $P<0.005$; Fig. 4], with an ED_{50} value (95% confidence limits) of 6.45 (2.64–15.79) mg/kg. At 10 mg/kg, the compound induced a 59% reduction in preference as compared with vehicle treatment ($P<0.05$).

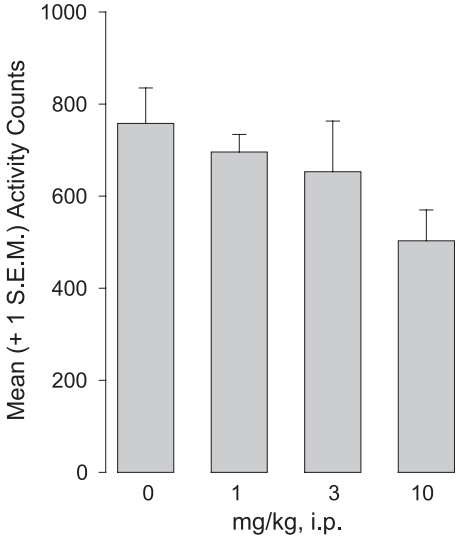

Fig. 3. Effect of SR141716A on locomotor activity in male rats habituated to the test environment. SR141716A was administered i.p, 30 min before the 10-min test session. $n=6$ per treatment.

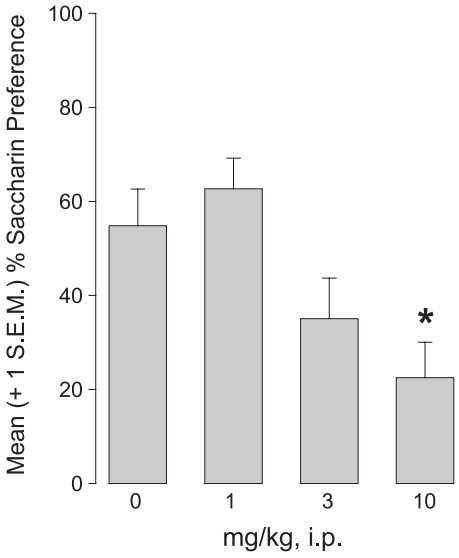

Fig. 4. Effect of SR141716A in a conditioned taste aversion (CTA) paradigm using male rats. Saccharin preference was expressed as the ratio of saccharin intake divided by total fluid intake, multiplied by 100, during the test session for CTA. SR141716A was administered i.p., 72 h before the 15-min test. $n=8$ per dose. *$P<0.05$ as compared with vehicle treatment.

4. Discussion

It has been suggested that the well-documented anorectic effect of the cannabinoid CB_1 receptor antagonist/inverse agonist SR141716A (Rinaldi-Carmona et al., 1994) results from an attenuating effect on feeding-related reward processes; either by (1) an attenuating effect of the compound on the orosensory characteristics of food (palatability hypothesis), or by (2) an attenuation of the appetitive or incentive aspects of feeding behavior (appetite hypothesis; Higgs et al., 2003; Kirkham and Williams, 2001b). The present study tested directly the latter hypothesis, using a limited-access food-maintained paradigm, and investigated to what extent drug-induced effects on locomotor activity and drug-induced aversion, possibly confound or contribute to the observed anorectic effects of the compound. As it was found that the compound almost equipotently suppresses food-maintained and electrical brain stimulation-maintained behavior, it is suggested that the anorectic effect of the compound results from an attenuating effect on the rewarding effect of food. However, because food-restriction did not potentiate suppression of food-maintained behavior by SR141716A, it can be argued that the anorectic effect is not primarily due to an inhibition of the appetitive aspects of feeding behavior. In addition, as locomotor activity was not (or hardly) affected when assessed during the same 10-min time-interval as used in the operant paradigms, it is unlikely that the suppression of food-maintained behavior is a direct consequence of an

impairment in locomotor activity. However, as the compound also induced conditioned taste aversion, a contribution of aversive effects or anhedonia induced at higher doses cannot be excluded.

The finding that SR141716A dose-dependently suppresses operant food-maintained behavior in food-restricted rats is consistent with the observations reported by Freedland et al. (2000, 2003) and Pério et al. (2001) using slightly different operant procedures. In the present study and in the Freedland et al. (2000, 2003) studies, which both used standard food pellets as reinforcer, a similar anorectic potency (MED of 1 mg/kg, i.p.) was obtained; whereas in the Pério et al. (2001), which used highly palatable sweet food pellets, intake was only suppressed in the 3–10 mg/kg dose range. Nevertheless, it has been suggested that SR141716A more potently suppresses the intake of highly palatable ingesta, such as sweet food or liquid, than the intake of less preferred ingesta, such as standard laboratory food or water (Freedland et al., 2001; Simiand et al., 1998). Microstructural analysis of the suppressive effect of SR141716A on sucrose drinking (i.e., analysis of the number of bouts, bout duration, and intra-bout lick rate) also suggested that it results from an attenuation of the palatability of sucrose (Higgs et al., 2003). Rowland et al. (2001) reported that SR141716A reduces intake of a highly palatable sweet milk dessert (in male rats), as well as normal chow intake (in 24 h food-restricted female rats), at doses lower than 3 mg/kg, but the lack of dose–response studies in the former paradigm precluded an estimation of the relative potency. The absence of major potency differences in the operant studies with ingesta of different palatability (present study; Freedland et al., 2000, 2003; Pério et al., 2001) suggests that high palatability is not a prerequisite for an anorectic effect of the compound, at least under conditions of food-restriction. Clearly, more studies are needed to determine whether the suggested higher anorectic sensitivity of SR141716A towards highly palatable ingesta is a function of the feeding status of the subject. Interestingly, it has been reported that SR141716A also reduces the intake of alcohol(ic beverages) in rodents (e.g., Arnone et al., 1997; Colombo et al., 1998b; Freedland et al., 2001; Gallate and McGregor, 1999), but it is not yet clear whether this effect is specific to (the palatability of) alcohol, or the consequence of a general suppressive effect on ingestive behavior. Thus, in alcohol-preferring sP rats, Colombo et al. (1998b) reported that SR141716A affects alcohol consumption in a slightly more potent manner than food consumption (at least when assessed during a relatively long time-interval); whereas in alcohol-preferring cAA rats, alcohol- and food intake appeared to be equipotently suppressed by SR141716A when assessed during a 12-h time-interval (unpublished data). In an elegant study, Gallate and McGregor (1999) found that SR141716A more effectively suppresses operant intake of beer (containing 4.5% ethanol) as compared with intake of near-beer (containing $<0.5\%$ ethanol), suggesting that the compound has a specific anti-alcohol effect.

The present study clearly demonstrates that food-restriction does not potentiate the suppressive effect of SR141716A on the consumption of normal food pellets (in fact, the ED_{50} value was slightly lower under free-feeding conditions, as compared with food-restriction conditions). From previous studies it was already clear that the introduction of food-restriction is not a prerequisite for the occurrence of an anorectic effect of the compound (e.g., Colombo et al., 1998a; Rowland et al., 2001; Simiand et al., 1998). However, only one preliminary study specifically tested whether food-restriction potentiates the anorectic potency/efficacy of SR141716A (Kirkham and Williams, 2001b). In that study it was found that SR141716A suppresses the intake of bland laboratory chow only under conditions of restricted food availability. This led the authors to conclude that the anorectic effect of SR141716A, at least partly, results from an inhibitory effect on appetite. Although many experimental details of that study were not reported (i.e., dose range tested), it is likely that the palatability of the food pellets used in the present study is higher than that of bland laboratory chow, and that the occurrence of a food-restriction effect (appetite effect predominating) can be counteracted if the palatability of the ingesta is high enough (palatability effect predominating).

When tested in the same 10-min, fixed ratio 10 operant paradigm, SR141716A was found to suppress intracranial self-stimulation with a potency which was only slightly lower than the anorectic potency in food-restricted male rats (ED_{50}: 4.5 mg/kg versus 2.5 mg/kg, respectively; difference not statistically different). The stimulation parameters of the intracranial self-stimulation paradigm were manipulated in such a manner that the resulting response rate approached that of the food-maintained paradigm (food-restriction in male rats); thereby minimizing possible confounding factors related to baseline level of responding. In a different assay, optimized for the detection of specific effects on reward threshold, Deroche-Gamonet et al. (2001) and Arnold et al. (2001) found that SR141716A decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. This effect was considered to be specific, as it appeared to be unaffected by possible effects on motor capabilities (for discussion and references, see Deroche-Gamonet et al., 2001). In the present study, SR141716A failed to affect locomotor activity (unless, perhaps, at the relatively high dose of 10 mg/kg, which tended to reduce activity counts), when assessed in the same dose range and same time-interval as in the operant paradigms (see also Freedland et al., 2000). Therefore it is concluded that the suppression of food- and electrical brain stimulation-maintained behavior is not confounded by an impairment in locomotor activity. As the present study demonstrates that the effect on electrical brain stimulation occurs in the same dose range which induces an anorectic effect (i.e., 1–10 mg/kg), it can be suggested that the latter effect results from a drug-induced attenuating effect on feeding-related reward processes. The finding that SR141716A attenuates the rewarding effect of

food, as assessed in a conditioned place preference paradigm, is also consistent with this suggestion (Chaperon et al., 1998).

In the final experiment, it was found that SR141716A dose-dependently attenuates saccharin preference in a standard conditioned taste aversion paradigm; suggesting that the compound may have aversive properties. Although the potency of SR141716A to induce a conditioned taste aversion was significantly lower than its anorectic potency (about 2.5- to 10-fold less potent, in terms of MED and ED_{50}, respectively), it opens the possibility that the anorectic effect induced at relatively high doses is, at least partly, due to drug-induced aversive effects, such as emesis or malaise (for references and discussion, see De Vry et al., 2000; De Vry and Schreiber, 2000). Although Chaperon et al. (1998) reported that the compound did not induce a conditioned place aversion (or preference), it should be noted that the highest dose tested in their study was 3 mg/kg (i.p.). As the presently observed conditioned taste aversion was only significant at 10 mg/kg, it remains possible that the presumed aversive properties become only observable at this high dose. Although it cannot be excluded that such high doses of SR141716A interact with other molecular targets than cannabinoid CB_1 receptors (for discussion, see Arnold et al., 2001), it should be realized that cannabinoid CB_1 receptors have a high level of spare receptors (i.e., high receptor reserve; Gifford et al., 1999), which implies that high doses of an antagonist are needed to fully block the endocannabinoid system (see also De Vry, 2002). Moreover, in the light of the well-known anti-emetic properties of cannabinoid CB_1 receptor agonists (Kalso, 2001), it is conceivable that SR141716A, which behaves as an inverse agonist at these receptors, is able to induce emesis. Indeed, in least shrews the compound was reported to induce vomiting (Darmani, 2001), and in rats administration of SR141716A (2.5 mg/kg, i.p.) was found to potentiate lithium-induced conditioned rejection reactions (Parker et al., 2003).

In conclusion, the present data are consistent with the suggestion that an attenuation of the rewarding properties of food contributes to the anorectic effect of SR141716A. The possibility that compound effects on appetitive aspects of feeding behavior are dependent on the palatability of the ingesta, and the role of drug-induced aversion, malaise or anhedonia, should be investigated further.

References

Arnold, J.C., Hunt, G.E., McGregor, I.S., 2001. Effects of the cannabinoid receptor agonist CP 55,940 and the cannabinoid receptor antagonist SR 141716 on intracranial self-stimulation in Lewis rats. Life Sci. 70, 97–108.

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

Chaperon, F., Soubrié, P., Puech, A.J., Thiébot, M.-H., 1998. Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. Psychopharmacology 135, 324–332.

Colombo, G., Agabio, R., Diaz, G., Lobina, C., Reali, R., Gessa, G.L., 1998a. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. Life Sci. 63, PL113–PL117.

Colombo, G., Agabio, R., Fà, M., Guano, L., Lobina, C., Loche, A., Reali, R., Gessa, G.L., 1998b. Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR-141716. Alcohol Alcohol. 33, 126–130.

Darmani, N.A., 2001. Delta-9-tetrahydrocannabinol and synthetic cannabinoids prevent emesis produced by the cannabinoid CB1 receptor antagonist/inverse agonist SR-141716A. Neuropsychopharmacology 24, 198–203.

De Beun, R., Lohmann, A., Schneider, R., De Vry, J., 1996. Ethanol intake-reducing effects of ipsapirone in rats are not due to simple stimulus substitution. Pharmacol. Biochem. Behav. 53, 891–898.

Deroche-Gamonet, V., Le Moal, M., Piazza, P.V., Soubrié, P., 2001. SR141716, a CB1 receptor antagonist, decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. Psychopharmacology 157, 254–259.

De Vry, J., 2002. Behavioral pharmacology of cannabinoids in animals. Eur. Neuropsychopharmacol. 12 (S3), S129.

De Vry, J., Schreiber, R., 2000. Effects of selected serotonin 5-HT_1 and 5-HT_2 receptor agonists on feeding behavior: possible mechanisms of action. Neurosci. Biobehav. Rev. 24, 341–353.

De Vry, J., Eckel, G., Kuhl, E., Schreiber, R., 2000. Effects of serotonin 5-HT_1 and 5-HT_2 receptor agonists in a conditioned taste aversion paradigm in the rat. Pharmacol. Biochem. Behav. 66, 797–802.

De Vry, J., Schreiber, R., Daschke, A., Jentzsch, K.R., 2003. Effects of serotonin 5-$HT_{1/2}$ receptor agonists in a limited-access operant food intake paradigm in the rat. Eur. Neuropsychopharmacol. 13, 337–345.

Di Marzo, V., Goparaju, S.K., Wang, L., Liu, J., Bátkai, S., Járai, Z., Fezza, F., Miura, G.I., Palmiter, R.D., Sugiura, T., Kunos, G., 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 410, 822–825.

Freedland, C.S., Poston, J.S., Porrino, L.J., 2000. Effects of SR141716A, a central cannabinoid receptor antagonist, on food-maintained responding. Pharmacol. Biochem. Behav. 67, 265–270.

Freedland, C.S., Sharpe, A.L., Samson, H.H., Porrino, L.J., 2001. Effects of SR141716A on ethanol and sucrose self-administration. Alcohol., Clin. Exp. Res. 25, 277–282.

Freedland, C.S., Whitlow, C.T., Smith, H.R., Porrino, L.J., 2003. Functional consequences of the acute administration of the cannabinoid receptor antagonist, SR141716A, in cannabinoid-naive and -tolerant animals: a quantitative 2-[^{14}C]deoxyglucose study. Brain Res. 962, 169–179.

Gallate, J.E., McGregor, I.S., 1999. The motivation for beer in rats: effects of ritanserin, naloxone and SR 141716. Psychopharmacology 142, 302–308.

Gifford, A.N., Bruneus, M., Gatley, S.J., Lan, R., Makriyannis, A., Volkow, N.D., 1999. Large receptor reserve for cannabinoid actions in the central nervous system. J. Pharmacol. Exp. Ther. 288, 478–483.

Higgs, S., Williams, C.M., Kirkham, T.C., 2003. Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after Δ^9-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. Psychopharmacology 165, 370–377.

Kalso, E., 2001. Cannabinoids for pain and nausea: some evidence but is there any need? BMJ 323, 2–3.

Kirkham, T.C., Williams, C.M., 2001a. Synergic effects of opioid and cannabinoid antagonists on food intake. Psychopharmacology 153, 267–270.

Kirkham, T.C., Williams, C.M., 2001b. Endogenous cannabinoids and appetite. Nutr. Res. Rev. 14, 65–86.

Kling-Petersen, T., Svensson, K., 1993. A simple computer-based method for performing and analyzing intracranial self-stimulation experiments in rats. J. Neurosci. Methods 47, 215–225.

König, J.F.R., Klippel, R.A., 1963. The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem Williams and Wilkins, Baltimore.

Parker, L.A., Mechoulam, R., Schlievert, C., Abbott, L., Fudge, M.L., Burton, P., 2003. Effects of cannabinoids on lithium-induced conditioned rejection reactions in a rat model of nausea. Psychopharmacology 166, 156–162.

Pério, A., Barnouin, M.-C., Poncelet, M., Soubrié, P., 2001. Activity of SR141716 on post-reinforcement pauses in operant responding for sucrose reward in rats. Behav. Pharmacol. 12, 641–645.

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, J.C., Le Fur, G., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Rowland, N.E., Mukherjee, M., Robertson, K., 2001. Effects of the cannabinoid receptor antagonist SR 141716, alone and in combination with dexfenfluramine or naloxone, on food intake in rats. Psychopharmacology 159, 111–116.

Simiand, J., Keane, M., Keane, P.E., Soubrié, P., 1998. SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. Behav. Pharmacol. 9, 179–181.