Behavioral effects of cannabinoids show differential sensitivity to cannabinoid receptor blockade and tolerance development

J. De Vry, K. R. Jentzsch, E. Kuhl and G. Eckel

This study compared the potency and efficacy of the cannabinoids Δ^9-tetrahydrocannabinol (Δ^9-THC), HU-210, WIN 55,212-2 and CP 55,940 in suppressing food-reinforced operant behavior, increasing reaction latency in a hot-plate test and inducing hypothermia, and tested whether these behavioral effects induced by CP 55,940 showed differential sensitivity to the cannabinoid CB_1 receptor antagonist SR141716A, and to tolerance development. After acute i.p. administration to rats, operant behavior was more potently affected than reaction latency and body temperature, but the order of potency of the different drugs was similar across the tests: HU-210<CP 55,940<WIN 55,212-2=Δ^9-THC. SR141716A blocked the hypothermic and analgesic effects more potently/efficiently than the response-rate suppressive effect of CP 55,940. After repeated administration of CP 55,940, the extent and speed of tolerance development was most pronounced in the hypothermia test, and least pronounced in the operant test. It is concluded that the more the behavioral effect induced by a cannabinoid receptor agonist is situated at the left-hand side of the dose-spectrum, the more the effect is resistant to blockade by a cannabinoid receptor antagonist and to the development of tolerance. The possible consequence of this observation for the therapeutic use of cannabinoids is discussed.

***Behavioural Pharmacology* 15:1–12 © 2004 Lippincott Williams & Wilkins.**

Behavioural Pharmacology 2004, **15**:1–12

Keywords: analgesia, body temperature, CP 55, 940, hot-plate test, HU-210, operant behavior, rat, SR141716A, Δ^9-tetrahydrocannabinol, WIN 55, 212-2

CNS Research, Bayer HealthCare, Wuppertal, Germany.

Correspondence to J. De Vry, Biomedical Research, Grünenthal, Zierglerstrasse 6, 52078, Aachen, Germany.
e-mail: Jean.DeVry@Grunenthal.de

Received 26 September 2003 **Accepted as revised** 18 November 2003

Introduction

The cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC, the major active constituent of *Cannabis sativa* L.; Gaoni and Mechoulam, 1964) and its highly potent derivative (−)-11-OH-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210; Järbe *et al.*, 1989); as well as nonclassical cannabinoids such as (−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol (CP 55,940; Johnson and Melvin, 1986), (*R*)-4, 5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6*H*-pyrrolo[3,2,1-ij] quinolin-6-one (WIN 55212-2; Compton *et al.*, 1992) and the recently discovered sulfonate derivatives BAY 38-7271 [(−)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate; Mauler *et al.*, 2002] and BAY 59-3074 (3-[2-cyano-3-(trifluoromethyl)phenoxy] phenyl-4,4,4-trifluoro-1-butanesulfonate; De Vry *et al.*, 2004c), all behave as mixed cannabinoid CB_1/CB_2 receptor agonists (for a review, see Pertwee, 1999). Although these compounds show differences in intrinsic activity at cannabinoid CB_1 receptors (the cannabinoid receptor subtype which is mainly expressed in the central nervous system; Moldrich and Wenger, 2000), they generally induce a qualitatively similar profile of behavioral activity across a broad dose-spectrum. Thus, at relatively high doses, classical and nonclassical cannabinoids induce in rodents a typical syndrome that consists of four major symptoms: analgesia (as measured by a hot-plate or tail flick test), hypoactivity, catalepsy and hypothermia (Martin *et al.*, 1991; Compton *et al.*, 1992; Pertwee, 1999). In addition, the above-mentioned compounds can function as discriminative stimuli in drug discrimination procedures, and, as far as investigated, they appear to induce cross-generalization to one another (Järbe *et al.*, 1989; Balster and Prescott, 1992; Wiley *et al.*, 1995; Pério *et al.*, 1996; De Vry and Jentzsch, 2002; Mauler *et al.*, 2002; De Vry *et al.*, 2004c). However, such discriminative effects can already be observed at relatively low doses, as compared to the doses generally needed to induce the typical cannabinoid behavioral syndrome. At doses that lie between those needed to induce the latter effects and the discriminative effects, these compounds were reported to have inhibitory effects on operant behavior, as assessed under various schedules of reinforcement (e.g. Carriero *et al.*, 1998; Lamb *et al.*, 2000; De Vry *et al.*, 2004a).

In general, the behavioral effects of cannabinoids can be attenuated, and eventually completely blocked, by selective cannabinoid CB_1 receptor antagonists, such as SR141716A (Rinaldi-Carmona *et al.*, 1994). Thus, the cannabinoid behavioral syndrome and the discriminative

0955-8810 © 2004 Lippincott Williams & Wilkins DOI: 10.1097/01.fbp.0000113330.49506.fc

effects induced by cannabinoids were found to be antagonized by pretreatment with SR141716A (e.g. Rinaldi-Carmona *et al.*, 1994; Wiley *et al.*, 1995; Mauler *et al.*, 2002; De Vry and Jentzsch, 2003; De Vry *et al.*, 2004c). However, it is unclear whether the various behavioral effects of cannabinoids are all equally sensitive to cannabinoid CB_1 receptor antagonism. Thus, it has been reported that the response-rate suppressive effect of cannabinoids in an operant procedure could not be completely blocked by SR141716A (De Vry *et al.*, 2004a; Järbe *et al.*, 2003).

Although the occurrence of tolerance to various behavioral effects of cannabinoids has been demonstrated, it is unclear whether tolerance develops at the same rate for each behavioral effect (for review, see Abood and Martin, 1992; Ameri, 1999). Thus, with respect to the symptoms contributing to the cannabinoid behavioral syndrome, rapid tolerance and cross-tolerance between cannabinoids was reported (Fan *et al.*, 1994); whereas apparently no tolerance develops to the appetite stimulant, anti-emetic and antihyperalgesic/anti-allodynic properties induced by relatively low doses of cannabinoids (De Vry *et al.*, 2004c; for review, see Felder and Glass, 1998).

It was the aim of the present study to investigate the relative potency of classical and nonclassical cannabinoids in inducing various behavioral effects under standardized conditions in the rat, and to investigate whether these effects were differentially sensitive to cannabinoid receptor blockade and to tolerance development. Thus, in a first experiment, dose–response curves were established with Δ^9-THC, HU-210, WIN 55,212-2 and CP 55,940 after acute i.p. administration, using operant behavior, hot-plate and body-temperature tests. Subsequently, it was tested whether SR141716A differentially affects the effect of CP 55,940 in the three tests. Finally, it was investigated to what extent tolerance develops differentially to the effects of CP 55,940 in the operant behavior, hot-plate and body-temperature tests.

Methods

Subjects

Male Wistar rats were purchased from Harlan–Winkelmann (HsdCpb: WU, Borchen, Germany). Body weight upon arrival at the laboratory ranged from 160 to 250 g. Rats were housed in groups of four, or individually (operant behavior test) in Makrolon® type 3 cages under a normal 12-hour light period (light on at 07.00 hours). The animals had unrestricted access to food and water, except for the operant behavior test, where food access was restricted (approximately 11.5–13 g/day, standard pellets; Ssniff Spezialdiäten GmbH, Soest, Germany). Room temperature and relative humidity were maintained at $21 \pm 1^{\circ}C$ and $55 \pm 5\%$, respectively. The procedures followed the guidelines for the use of animals, as given by the German government, and was approved by the local authorities (Regierungspräsidium Düsseldorf, Germany).

Operant behavior test

Experiments were performed in sound- and light-attenuated standard operant chambers (Coulborn Instruments, Lehigh Valley, Pennsylvania, USA). The chambers were equipped with two levers equidistant from a food tray between the levers. Food reinforcement (45 mg precision pellets, Bio-Serv, New Jersey, USA) was delivered by an automated food dispenser located outside of the chamber. Data collection and experimental contingencies were programmed using OPN software on a PC interfaced with the operant chamber. Ventilation and masking noise were provided by a fan mounted on the wall of the chamber. A white houselight was switched on during the sessions, which were conducted between 09.00 and 15.00 hours.

In general, the procedure described by De Vry *et al.* (2003) was followed. After initial shaping to press on the left lever for food reinforcement, the 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 (5 sessions/week, Monday through Friday). Pharmacological testing was started after reaching stable baselines of operant responding (about 800 responses/session). In a first series of experiments, the effect of acute administration of different cannabinoid receptor agonists on operant behavior was assessed. Different groups ($n = 6$–8 per group) were injected i.p. with HU-210 (0, 0.01, 0.03 and 0.1 mg/kg, and on a separate occasion, 0 and 0.003 mg/kg), CP 55,940 (0, 0.03, 0.1 and 0.3 mg/kg, and on a separate occasion, 0 and 0.001 mg/kg), WIN 55,212-2 (0, 0.3, 1 and 3 mg/kg) and Δ^9-THC (0, 1, 3 and 10 mg/kg), and tested 30 min later during a similar fixed-ratio 10, food-reinforced, 10-min session (except for HU-210: t – 120 min). For each compound, the different doses (and the respective vehicle) were tested only once (i.e. each of the four groups received a particular dose of the test compound or vehicle on the test day, usually performed once a week). Before each test, the rats were randomly assigned to the four groups and they were tested repeatedly with a washout period of at least 1 week between injections.

In a subsequent experiment, it was tested whether pretreatment with SR141716A reversed the response-rate suppressive effect of CP 55,940. Four groups of rats ($n = 8$ per group) were injected with either 1 mg/kg SR141716A (i.e. the maximal dose that was found to be devoid of a response-rate suppressive effect when tested alone in this paradigm; De Vry *et al.*, 2004b) or vehicle, followed 10 min later by an i.p. injection with 0.1 mg/kg CP 55,940 or vehicle, and the animals were tested during

a 10-min session, which started 30 min after the second injection.

In a final experiment, the effect of repeated administration of CP 55,940 on operant behavior was assessed. During four consecutive days, rats ($n = 7$–8 per group) were treated i.p. with CP 55,940 (0, 0.01, 0.03 and 0.1 mg/kg; each group always received the same treatment). Injections were given twice-daily, between 09.00 and 11.00 h and between 15.00 and 17.00 h, respectively (except for day 4, during which only one injection was given, between 09.00 and 11.00 h). Two 10-min test sessions were performed starting 30 and 60 min after the first injection of each day, respectively.

Body-temperature test

In general, the procedure described by Mauler *et al.* (2002) was followed. Measurement of core body temperature was performed esophageally with a digital thermocouple laboratory thermometer (Model BAT-12, Technical and Scientific Equipment GmbH, Bad Homburg, Germany) equipped with a temperature-measurement probe for esophageal use (Model RET-2, Technical and Scientific Equipment GmbH). In a first series of experiments, the effect of acute administration of the cannabinoid receptor agonists on body temperature was assessed. Five minutes after measurement of baseline body temperature, different groups ($n = 8$) were injected i.p. with HU-210 (0, 0.01, 0.03 and 0.1 mg/kg), CP 55,940 (0, 0.03, 0.1 and 0.3 mg/kg), WIN 55,212-2 (0, 2, 6 and 20 mg/kg) and Δ^9-THC (0, 1, 3 and 10 mg/kg), and tested again 30 and 60 min later (except for HU-210: $t - 60$ and 120 min). In the case of WIN 55,212-2, a second experiment was performed, in which the compound was administered i.p. (0, 1, 3, 10 and 30 mg/kg; $n = 8$ per group, except for the group injected with vehicle: $n = 12$), and the animals were tested 5 min before, and 5, 10, 20, 40 and 80 min after, administration. In a subsequent experiment, whether pretreatment with SR141716A reversed hypothermia induced by CP 55,940 was tested. Four groups of rats ($n = 8$–9 per group) were injected with either 0 or 1 mg/kg SR141716A, followed 10 min later by an i.p. injection with 0.3 mg/kg CP 55,940 or vehicle, and the animals were tested 5 min before the first injection, and 30 and 60 min after the second injection. SR141716A was tested at a dose of 1 mg/kg, as preliminary experiments indicated that 0.3 mg/kg already partially blocked hypothermia (as well as the reaction latency increase in the hot-plate test) induced by 0.3 mg/kg CP 55,940 (De Vry and Eckel, unpublished). In a final experiment, the effect of repeated administration of CP 55,940 on body temperature was assessed. During four consecutive days, rats ($n = 8$ per group) were treated with CP 55,940 (0, 0.1, 0.2 and 0.4 mg/kg, i.p.; each group always received the same treatment). Injections were given twice-daily, between 09.00 and 11.00 h and between 15.00 and 17.00 h, respectively (except for day 4, during which only one injection was given, between 09.00 and 11.00 h). The body-temperature test was performed after the first injection of each day. Measurements occurred 5 min before, and 30 and 60 min after, injection.

Hot-plate test

The hot-plate test was performed immediately after measurement of core body temperature (same rats), and therefore the same drug application conditions as described in the previous section were tested (i.e. acute dose–response experiments, antagonism experiment and subacute dose–response experiment; in the case of the second dose–response experiment with WIN 55,212-2 only, no hot-plate test was performed). The hot-plate apparatus (Model Cat. 7250, 25 × 25 cm, Ugo Basile, Comerio, Italy), equipped with a clear Plexiglas cylinder, was gradually heated to 52°C, about 30 min before the start of the experiment. The device guaranteed a temperature stability with a variation of 0.2°C. Reaction latency time (in s) was scored with a foot-operated stopwatch. After being placed on the hot plate, animals were observed for the occurrence of vocalization, licking or lifting of the hindpaw, or jumping. As soon as one of these symptoms occurred, the reaction latency was recorded and the animals were taken from the hot plate. If no reaction occurred within 40 s, animals were taken from the hot plate and given the latency score of 40 s (cut-off). The hot plate was wiped clean before testing the next animal.

Drugs

HU-210 [(−)-11-OH-Δ^8-tetrahydrocannabinol-dimethylheptyl], CP 55,940 {(−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol}, and SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride] were synthesized by the Medical Chemistry Department of Bayer HealthCare (Wuppertal, Germany). Δ^9-THC [(−)-Δ^9-tetrahydrocannabinol] and WIN 55,212-2 [(*R*)-4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6*H*-pyrrolo[3,2,1-*ij*]quinolin-6-one] were purchased from Sigma-Aldrich Chemie (Steinheim, Germany) and RBI (Natick, Massachusetts, USA), respectively. Compounds were administered in a solvent containing 2.5–5% Solutol® HS 15 (12-hydroxystearic-acid ethoxylate; BASF AG, Ludwigshafen, Germany) and 2.5–5% ethanol (ethanol absolute, 99.8%; Riedel-de Haën, Seelze, Germany), and distilled water. Injections were given i.p. in an application volume of 2 ml/kg (operant behavior test) or 5 ml/kg body weight (other tests).

Data analysis

Data were analyzed by one-factor (dose–response experiments), or two-factor (antagonism experiments) ANOVA, followed, when appropriate, by Tukey's *post-hoc*

comparison. In the case of HU-210 and CP 55,940 in the operant paradigm, additional tests with particular doses (and the corresponding vehicle) were needed in order to obtain the complete dose–response curve. In that case, data obtained with vehicle were pooled (as there was no evidence for a statistical difference between the first and second test with vehicle) for data analysis and graphical presentation, and only the minimal effective dose range was shown (eventually leading to the omission of doses higher than the maximally effective dose). For graphical presentation, efficacy and ED_{50} value calculation, drug effects were also expressed as percentage reduction in number of responses, as compared with vehicle treatment. For graphical presentation of the body temperature data, results were expressed as mean absolute body temperature, or as temperature change in °C relative to baseline value. For efficacy and ED_{50} value calculation, a temperature reduction of 1.5°C (as compared with baseline control) was arbitrarily defined as 100% effect. For graphical presentation, efficacy and ED_{50} value calculation of the hot-plate assay results, efficacy was expressed as percentage of the maximal possible effect (%MPE) given by the following equation:

$$\%\text{MPE} = 100\% \frac{\text{test drug latency} - \text{vehicle latency}}{\text{cutoff time} - \text{vehicle latency}}$$

Least-square linear regression analysis was used to estimate ED_{50} values and the corresponding 95% confidence limits (CL) after log-probit conversion of the data. ED_{50} values with nonoverlapping CL limits were considered to be significantly different.

Results

Operant behavior test

Throughout the experiments, baseline values remained stable. Mean number of responses on the training sessions of the day preceding each test day ranged from 700 to 910 responses, and, for all tested compounds, the number of responses returned to baseline values within 2 days following the test sessions (except for the 0.03 and 0.1 mg/kg doses of HU-210, where 3 and 4 days were required, respectively). Each cannabinoid dose-dependently suppressed operant responding during the 10-min test session (Fig. 1, left panel, outcome of the respective ANOVAs in Table 1, outcome of *post-hoc* tests in Fig. 1). As estimated by the respective ED_{50} values (Table 1), the following order of potency was obtained: HU-210 < CP 55,940 < WIN 55,212-2 = Δ^9-THC. In terms of efficacy, no difference between the compounds was obtained, as they all completely suppressed responding at the higher doses tested. On the non-treated baseline session preceding pharmacological testing, the mean number (± SEM) of responses in the different groups ranged from 700 (70) to 820 (80), 720 (80) to 870 (130), 780 (60) to 860 (70), and 810 (80) to 910 (50), in the groups tested with HU-210, CP 55,940, WIN 55,212-2 and Δ^9-THC, respectively, and was not significantly different between the groups of each experiment.

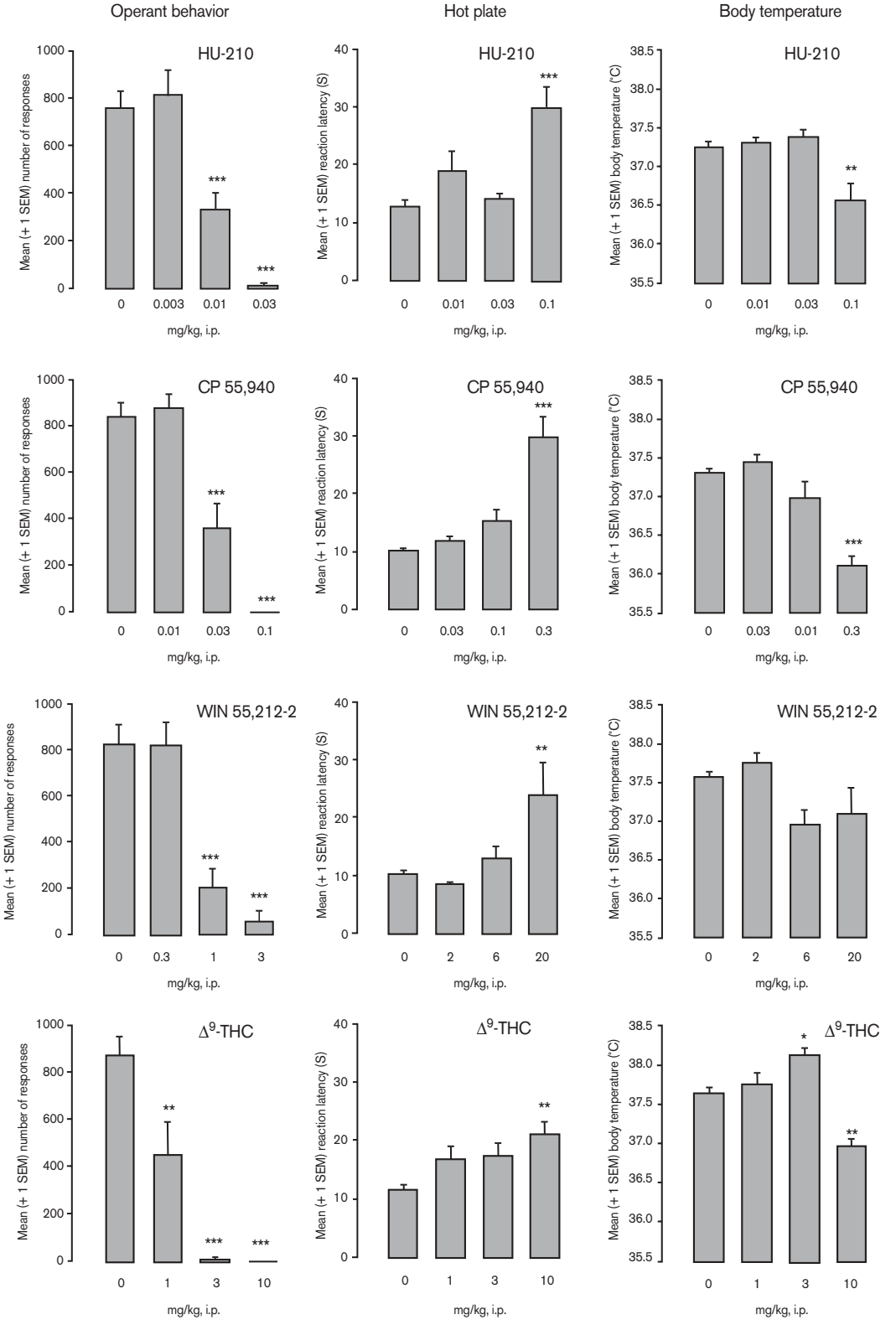
The effect of pretreatment with SR141716A on response suppression induced by CP 55,940 is shown in the left panel of Fig. 2. ANOVA indicated a main effect of 0.1 mg/kg CP 55,940 [$F(1,28) = 139.72$, $P < 0.001$]. There was, however, no evidence for a main effect of SR141716A [$F(1,28) = 0.08$, NS], nor for an interaction between the two compounds [$F(1,28) = 2.16$, NS]. This indicates that 1 mg/kg SR141716A did not antagonize response suppression induced by CP 55,940. On the non-treated baseline session, the mean number (± SEM) of responses in the different groups ranged from 890 (50) to 910 (70), and was not significantly different between the groups.

The effect of repeated administration of CP 55,940 on operant behavior is shown in the left panel of Fig. 3. Acute administration of CP 55,940 (day 1) dose-dependently and completely suppressed operant responding (ED_{50} values and outcome of the respective ANOVAs in Table 2, outcome of *post-hoc* tests in Fig. 3). Comparison of the dose–response curve obtained after 7 applications (day 4) with that obtained after single application (day 1) indicated that repeated administration of the compound hardly affected its potency and efficacy to suppress operant behavior, as assessed 30 and 60 min after injection (data obtained on days 2 and 3 are not shown, but respective ED_{50} values, maximal efficacy and outcome of ANOVAs are shown in Table 2). On the non-treated baseline session before the start of drug administration, the mean number (± SEM) of responses in the different groups ranged from 780 (50) to 900 (40), and was not significantly different between the groups.

Hot-plate test

Effects obtained with the cannabinoids in the hot-plate assay are shown in the middle panel of Fig. 1 (30 min post-application, as the data obtained after 60 min were similar and therefore are not shown; in the case of HU-210, data shown represent the results obtained after 120 min, as the compound had a relatively slow onset of action). The four cannabinoids increased reaction latency (ED_{50} values and outcome of the respective ANOVAs in Table 1, outcome of *post-hoc* tests in Fig. 1), with a similar order of potency as in the operant behavior test, but an absolute potency which was about 10–20 times lower (as assessed at the same injection–test time interval). In addition, the slope of the dose–response curve appeared to be less steep in the case of Δ^9-THC. Mean (± SEM) baseline reaction latency in seconds, as measured 5 min before injection, ranged from 8.9 (0.6) to 9.8 (0.7), 9.8 (0.6) to 10.9 (0.4), 8.5 (0.9) to 9.9 (0.7), and 10.6 (0.6) to 12.1 (0.9), in the groups tested with HU-210, CP 55,940, WIN 55,212-2 and Δ^9-THC, respectively, and was not significantly different for each experiment.

Fig. 1



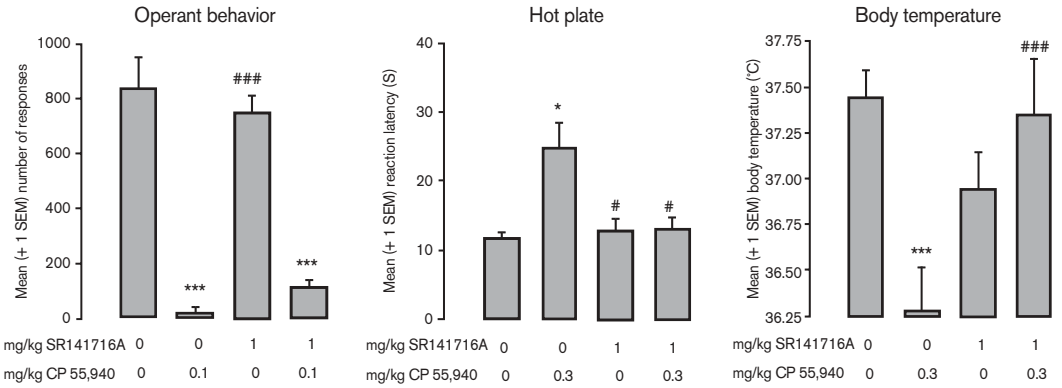
Effects of acute administration of HU-210, CP 55,940, WIN 55,212-2 and Δ^9-THC on operant behavior, reaction latency in a hot-plate test and body temperature. In the operant behavior test, rats were trained to respond on a fixed-ratio 10 schedule of food reinforcement during daily 10-min sessions. Data shown represent the effects obtained 30 min after administration (except HU-210: t – 120 min). n=8 per group (operant behavior: n=6–8 per group). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ as compared with vehicle treatment.

Table 1 Effect of acute administration of cannabinoids on food-reinforced operant behavior, reaction latency in a hot-plate test and body temperature

Compound	Test	Time[a]	ED_{50} (95% CL)[b]	ANOVA
HU-210	Operant	120	0.009 (0.005–0.016)	$F(3,36)=72.63$, $P<0.001$
	Hot plate	60	0.31 (0.01–6.56)	$F(3,28)=4.39$, $P<0.05$
		120	0.082 (0.045–0.148)	$F(3,28)=10.88$, $P<0.001$
	Temperature	60	>0.1	$F(3,28)=6.81$, $P<0.001$
		120	0.10 (0.06–0.18)	$F(3,28)=9.65$, $P<0.001$
CP 55,940	Operant	30	0.030 (0.017–0.052)	$F(3,34)=66.71$, $P<0.001$
	Hot plate	30	0.22 (0.10–0.47)	$F(3,28)=22.96$, $P<0.001$
		60	0.27 (0.10–0.72)	$F(3,28)=16.74$, $P<0.001$
	Temperature	30	0.17 (0.10–0.31)	$F(3,28)=24.25$, $P<0.001$
		60	0.15 (0.08–0.26)	$F(3,28)=44.26$, $P<0.001$
WIN 55,212-2	Operant	30	1.06 (0.49–2.31)	$F(3,27)=56.31$, $P<0.001$
	Hot plate	30	22.16 (8.21–59.79)	$F(3,28)=6.09$, $P<0.01$
		60	32.3 (11.90–87.37)	$F(3,28)=4.52$, $P<0.01$
	Temperature	30	>20	$F(3,28)=3.44$, $P<0.05$
		60	>20	$F(3,28)=1.65$, $P>0.05$
Δ^9-THC	Operant	30	1.02 (NC)	$F(3,23)=26.80$, $P<0.001$
	Hot plate	30	>10	$F(3,28)=4.60$, $P<0.01$
		60	18.55 (3.43–100.39)	$F(3,28)=6.18$, $P<0.01$
	Temperature	30	>10	$F(3,28)=20.76$, $P<0.001$
		60	>10	$F(3,28)=13.07$, $P<0.001$

[a] Injection–test interval in minutes.
[b] Doses in mg/kg. CL, confidence limits, NC, not computable.

Fig. 2

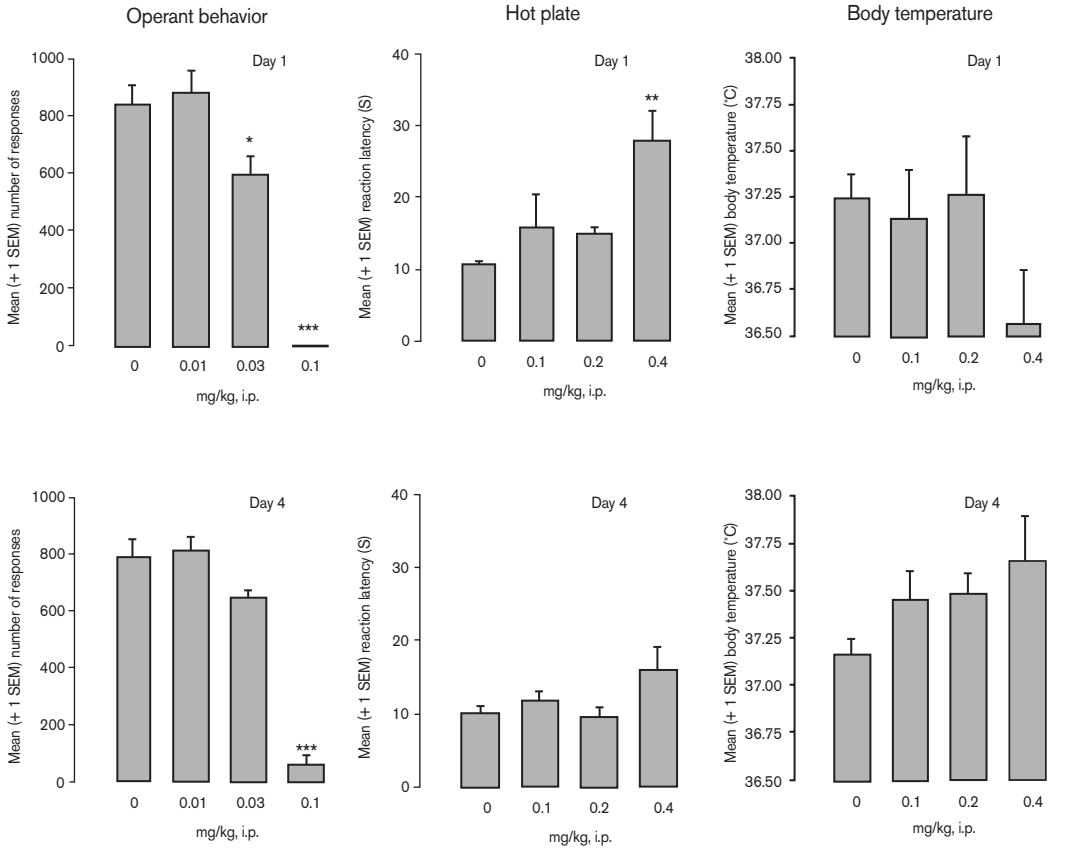


Effects of pretreatment with SR141716A (dose, shown below the figures, in mg/kg, i.p.; *t* – 40 min) on the effect of CP 55,940 (dose in mg/kg, i.p.; *t* – 30 min) on operant behavior, reaction latency in a hot-plate test and body temperature; $n=8$–9 per group. * $P<0.05$, *** $P<0.001$ as compared with vehicle/vehicle treatment, # $P<0.05$, ### $P<0.001$ as compared with vehicle/CP 55,940 treatment.

The effect of pretreatment with SR141716A on the increase in response latency induced by CP 55,940 is shown in the middle panel of Fig. 2 (as assessed 30 min after the second administration; data obtained after 60 min were similar and therefore are not shown). ANOVA indicated a main effect of CP 55,940 [30 min: $F(1,31) = 6.09$, $P < 0.05$; 60 min: $F(1,31) = 16.16$, $P < 0.001$] and SR141716A [30 min: $F(1,31) = 5.53$, $P < 0.05$; 60 min: $F(1,31) = 18.78$, $P < 0.001$], as well as a significant interaction between the two drugs [30 min: $F(1,31) = 5.29$, $P < 0.05$; 60 min: $F(1,31) = 14.59$, $P < 0.001$]. SR141716A completely antagonized the increase in reaction latency induced by CP 55,940 (30 min: $P < 0.05$, 60 min: $P < 0.001$). Mean (± SEM) baseline reaction latency in seconds ranged from 9.6 (0.6) to 10.6 (0.8), and was not significantly different between the groups.

The effect of repeated administration of CP 55,940 in the hot-plate test is shown in the middle panel of Fig. 3 (as assessed 60 min after administration, data obtained after 30 min were similar and therefore are not shown). Acute administration with CP 55,940 (day 1) significantly increased reaction latency (ED_{50} values and outcome of the respective ANOVAs in Table 2, outcome of *post-hoc* tests in Fig. 3). Comparison of the dose–response curves obtained after seven applications (day 4) and one application (day 1), however, indicated that repeated

Fig. 3



Effects of repeated treatment with CP 55,940 on operant behavior, reaction latency in a hot-plate test and body temperature. Rats were injected twice-daily with either 0, 0.01, 0.03 and 0.1 mg/kg (former test), or 0, 0.1, 0.2 and 0.4 mg/kg (latter tests) on four consecutive days, and were tested 30 and 60 min after the first injection of each day. The figure shows the data obtained on the first and last day of application (i.e. after 1 and 7 applications), 30 min after application, in the case of operant behavior, and 60 min after application, in the case of the two other tests. $n=7$–8 per group. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ as compared with vehicle treatment.

administration of the compound induced tolerance to the reaction latency increasing effect of the compound (effect no longer statistically significant on days 2 and 4). Mean (± SEM) baseline reaction latency in seconds ranged from 8.5 (0.7) to 9.4 (0.6), and was not significantly different between the groups.

Body-temperature test

Effects obtained with the cannabinoids in the body-temperature assay are shown in the right panel of Fig. 1 (30 min post-application, as the data obtained after 60 min were similar and therefore are not shown; in the case of HU-210, data shown represent the results obtained after 120 min, due to its slow onset of action). Each compound induced hypothermia (data and outcome of *post-hoc* tests in the right panel of Fig. 1, ED_{50} values and outcome of the respective ANOVAs in Table 1). Although WIN 55,212-2 failed to induce a statistically significant hypothermia, a clear trend for such an effect was present at the 6 and 20 mg/kg doses. In the additional dose–response study including a higher dose of this compound, the hypothermic effect was maximal 40 min after administration, and amounted to –0.1, –0.3, –0.5 and –1.6°C, after administration of 1, 3, 10 and 30 mg/kg WIN 55,212-2, respectively [data not shown; $F(4,39) = 10.77$, $P < 0.001$, *post-hoc* analysis indicated that the effect was statistically significant only at the highest dose tested, $P < 0.001$]. Absolute and relative potency were similar in the hot-plate test and the hypothermia test (it should be recognized, however, that the 100% effect was defined arbitrarily in both tests). Mean (± SEM) baseline body temperature ranged from 36.9 (0.1) to 37.1 (0.1), 36.8 (0.1) to 36.9 (0.1), 37.0 (0.1) to 37.2 (0.1), and 37.1 (0.04) to 37.3 (0.1)°C, in the groups tested with HU-210, CP 55,940, WIN 55,212-2 and Δ^9-THC, respectively, and was not significantly different for each experiment.

Table 2 Effect of repeated administration of CP 55,940 on operant behavior, reaction latency in a hot-plate test and body temperature. Rats were injected twice-daily on four consecutive days, and tested 30 and 60 min after the first injection of each day

Test	Day	Time[a]	Efficacy[b]	ED_{50} (95% CL)[c]	ANOVA
Operant	1	30	100%	0.034 (0.020–0.059)	$F(3,27)=95.76$, $P<0.001$
		60	99%	0.026 (0.013–0.049)	$F(3,27)=69.81$, $P<0.001$
	2	30	100%	0.034 (0.020–0.058)	$F(3,27)=56.90$, $P<0.001$
		60	91%	0.042 (0.022–0.082)	$F(3,27)=60.19$, $P<0.001$
	3	30	96%	0.038 (0.021–0.069)	$F(3,27)=102.67$, $P<0.001$
		60	96%	0.037 (0.021–0.067)	$F(3,27)=40.51$, $P<0.001$
	4	30	92%	0.044 (0.023–0.083)	$F(3,27)=85.97$, $P<0.001$
		60	85%	0.048 (0.029–0.080)	$F(3,27)=40.22$, $P<0.001$
Hot plate	1	30	62%	0.32 (0.20–0.52)	$F(3,28)=6.33$, $P<0.01$
		60	58%	0.42 (0.18–0.99)	$F(3,28)=5.92$, $P<0.01$
	2	30	28%	>0.4	$F(3,28)=2.16$, $P>0.05$
		60	20%	>0.4	$F(3,28)=2.05$, $P>0.05$
	3	30	37%	>0.4	$F(3,28)=6.12$, $P<0.01$
		60	41%	>0.4	$F(3,28)=8.59$, $P<0.001$
	4	30	30%	>0.4	$F(3,28)=7.49$, $P<0.001$
		60	19%	>0.4	$F(3,28)=2.64$, $P>0.05$
Temperature	1	30	−0.6°C	>0.4	$F(3,28)=3.24$, $P<0.05$
		60	−1.1°C	0.33 (0.15–0.70)	$F(3,28)=1.61$, $P>0.05$
	2	30	−0.5°C	>0.4	$F(3,28)=1.98$, $P>0.05$
		60	−0.5°C	>0.4	$F(3,28)=1.71$, $P>0.05$
	3	30	−0.6°C	>0.4	$F(3,28)=5.21$, $P<0.01$
		60	−0.6°C	>0.4	$F(3,28)=3.88$, $P<0.05$
	4	30	−0.2°C	>0.4	$F(3,28)=0.54$, $P>0.05$
		60	+0.4°C	>0.4	$F(3,28)=1.61$, $P>0.05$

[a] Injection–test interval in minutes.
[b] Maximal effect across tested dose range.
[c] Doses in mg/kg. CL, confidence limits.

The effect of pretreatment with SR141716A on hypothermia induced by CP 55,940 is shown in the right panel of Fig. 2 (30 min after the second administration, data obtained after 60 min were similar and therefore are not shown). There was a main effect of CP 55,940 [30 min: $F(1,31) = 24.50$, $P < 0.001$; 60 min: $F(1,31) = 23.48$, $P < 0.001$] and SR141716A [only significant at 60 min: $F(1,31) = 6.64$, $P < 0.05$; 30 min: $F(1,31) = 2.03$, NS], and a significant interaction effect [30 min: $F(1,31) = 5.40$, $P < 0.05$; 60 min: $F(1,31) = 11.82$, $P < 0.01$]. SR141716A antagonized hypothermia induced by CP 55,940 (30 and 60 min: $P < 0.001$). Mean (±SEM) baseline body temperature ranged from 36.9 (0.04) to 37.0 (0.1)°C, and was not significantly different between the groups.

The effect of repeated administration of CP 55,940 on body temperature is shown in the right panel of Fig. 3 (as assessed 60 min after administration, data obtained after 30 min were similar and therefore are not shown). Although acute administration with CP 55,940 (day 1) failed to decrease body temperature significantly, a clear trend for a hypothermic effect was present (ED_{50} values and outcome of the respective ANOVAs in Table 2, outcome of *post-hoc* tests in Fig. 3). Comparison of the dose–response curves obtained after seven applications (day 4) and one application indicated that the trend for a hypothermic effect was completely abolished after repeated administration (in fact on day 4, there was a trend for a hyperthermic effect). Mean (±SEM) baseline body temperature in °C ranged from 37.1 (0.1) to 37.5 (0.1), and was not significantly different between the groups.

Discussion

The present study showed that classical cannabinoids, such as Δ^9-THC and HU-210, as well as nonclassical cannabinoids, such as CP 55,940 and WIN 55212-2, induce a qualitatively similar profile of activity in the three selected behavioral tests. Thus after acute i.p. administration, food-reinforced operant behavior was more potently affected by the four compounds, than body temperature and reaction latency in a hot-plate test. However, in each test the order of potency was similar: HU-210 < CP 55,940 < WIN 55,212-2 = Δ^9-THC (only, in the body-temperature test, Δ^9-THC appeared to be more potent than WIN 55,212-2). In addition, it was found that the cannabinoid CB_1 receptor antagonist SR141716A more potently/efficiently blocked the hypothermic and analgesic effects, than the response rate suppressive effect of CP 55,940, and that, after repeated administration of CP 55,940, the extent and speed of tolerance development was much more pronounced in the former two tests, than in the operant test.

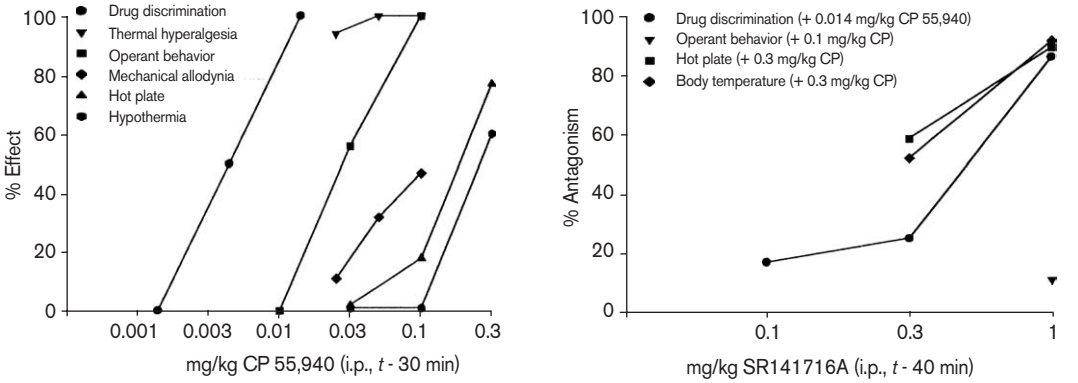
The finding that cannabinoids induce hypothermia and analgesia at relatively high doses has been well established (e.g. Compton *et al.*, 1992; Fan *et al.*, 1994; Pertwee, 2001; Mauler *et al.*, 2002), and supports the notion that cannabinoid CB_1 receptor agonists share the ability to induce a similar profile of effects, commonly referred to as the cannabinoid behavioral syndrome (Martin *et al.*,

1991). The fact that these compounds dose-dependently suppress food-reinforced operant behavior is also consistent with previous reports obtained in similar procedures (e.g. Carriero *et al.*, 1998; Lamb *et al.*, 2000; De Vry *et al.*, 2004a). As compared with the hot-plate and hypothermia test, the four compounds showed the same order of potency in the operant test, but, in general, their potency in the latter test was about 10–20 times higher. It was previously reported that, in drug discrimination procedures using these cannabinoids as training drugs, cross-generalization between them was consistently obtained, again with the same order of potency, but, in general, with ED_{50} values of generalization which were about 3–10 times lower than the respective ED_{50} values of response-rate suppression (for references, see Introduction). It is therefore concluded that the behavioral effects of cannabinoids extend across a wide dose-range (as exemplified with CP 55,940 in Fig. 4, left-hand panel).

Because the major difference in behavioral activity between these compounds relates to potency, rather than efficacy, it can be assumed that the behavioral profile of cannabinoid CB_1 receptor agonists is mainly determined by the compound's receptor affinity, rather than its intrinsic activity, at these receptors. This concept is reinforced by the finding that, despite its lower intrinsic activity at cannabinoid CB_1 receptors as compared to the other compounds (Petitet *et al.*, 1998; Mauler *et al.*, 2002), Δ^9-THC showed virtually the same level of activity *in vivo* (except, perhaps, in the hot-plate test, where the dose–response curve of Δ^9-THC appeared to be somewhat less steep as compared to the curves obtained with the other compounds). At the molecular level, this phenomenon could be explained by the presence of a high degree of receptor reserve (or receptor–effector coupling). Indeed, Gifford *et al.* (1999) reported that there is a large receptor reserve for WIN 55,212-2-induced inhibition of locomotor activity (which, as assessed with CP 55,940 in rats, occurs in the same dose range as the suppression of operant behavior; De Vry and Kuhl, unpublished). According to this hypothesis, it can be postulated that, once the intrinsic activity of a cannabinoid receptor agonist surpasses a rather low, but critical, level, the compound will be able to produce a typical cannabinoid-like behavioral profile. The observation that the diverse behavioral effects of cannabinoids extend across such a wide dose-range can tentatively also be explained by the receptor reserve concept. Thus, it is predicted that the higher the potency of a particular cannabinoid-induced behavioral effect is, the higher will be the receptor reserve of the receptor population mediating the behavioral effect. This would imply that the level of receptor reserve is higher for the receptor population mediating the discriminative effects of cannabinoids, than for the receptor population mediating the hypothermic and analgesic effects (for further discussion, see De Vry and Jentzsch, 2003).

Although it was previously reported that most, if not all, of the behavioral effects of cannabinoids can be blocked by the selective cannabinoid CB_1 receptor antagonist SR141716A (Rinaldi-Carmona *et al.*, 1994), it remains unclear to what extent these effects are differentially sensitive to such blockade. Thus, whereas the cannabinoid-induced behavioral syndrome and discriminative effects are antagonized by SR141716A (e.g. Rinaldi-Carmona *et al.*, 1994; Wiley *et al.*, 1995; Mauler *et al.*, 2002), the response-rate suppressive effect of

Fig. 4



Overview of selected behavioral effects of CP 55,940 in rats (left panel), and their reversal by SR141716A (right panel). Effects induced by CP 55,940 were expressed as % generalization in animals trained to discriminate 0.014 mg/kg CP 55,940 from vehicle (drug discrimination; data adapted from De Vry and Jentzsch, 2003), % reduction in response rate (operant behavior), % increase in reaction latency (hot plate), % reversal of thermal hyperalgesia and mechanical allodynia in the chronic constriction injury model of neuropathic pain (data adapted from De Vry, 2002), and % reduction of body temperature (hypothermia), respectively. Antagonist effects induced by SR141716A were expressed as % reversal of the effect induced by a challenge dose of CP 55,940 (CP, defined as 100% effect dose).

cannabinoids in an operant procedure could not be completely blocked by SR141716A (De Vry *et al.*, 2004a; Järbe *et al.*, 2003). In the present study, pretreatment with 1 mg/kg SR141716A completely blocked the hypothermic and analgesic effect induced by a relatively high dose of CP 55,940 (0.3 mg/kg), but failed to affect the response rate suppressive effect induced by a lower dose (0.1 mg/kg). Indeed, comparison of the relative potency/efficacy of SR141716A to block the discriminative, response-rate suppressive, hypothermic and analgesic effects of CP 55,940 (Fig. 4, right-hand panel) indicates that the diverse behavioral effects of CP 55,940 are not equally sensitive to blockade by the antagonist.

In general, it appears that the more the behavioral effect induced by a cannabinoid receptor agonist is situated at the left side of the dose-spectrum, the more the effect is resistant to blockade by SR141716A. Thus, whereas 0.3 mg/kg SR141716A hardly attenuates the discriminative effects of 0.014 mg/kg CP 55,940, the same dose of SR141716A already partially antagonizes the antinociceptive and hypothermic effects induced by 0.1 mg/kg CP 55,940. Interestingly, suppression of operant behavior by CP 55,940 appears particularly resistant against blockade by SR141716A (even more resistant than the discriminative effect of CP 55,940). However, this observation may be complicated by the fact that SR141716A also dose-dependently suppresses operant behavior when assessed under the same experimental conditions (De Vry *et al.*, 2004a, b).

It has been proposed that this profile of activity of SR141716A in the operant procedure may reflect partial agonist properties of this compound (De Vry and Jentzsch, 2003; De Vry *et al.*, 2004a). Alternatively, the apparent high resistance of CP 55,940 to antagonism by SR141716A in the operant procedure could be related to an interaction of different behavioral mechanisms. Thus, it has been argued that the response-rate suppressive effect of SR141716A results from an attenuating effect on food reward (for discussion, see De Vry *et al.*, 2004b); whereas the response-rate suppressive effect of the cannabinoids results from a nonspecific sedative of motor disruptive effect (Carriero *et al.*, 1998). Although cannabinoids increase appetite and induce hyperphagic effects (Kirkham and Williams, 2001), it is likely that these effects are masked or overridden by these nonspecific motor effects in the operant procedure, resulting in response-rate suppression. It is conceivable that SR141716A blocks the latter effects of the cannabinoids, and thereby unmasks the appetite stimulating effect of the cannabinoids. Combined administration of SR141716A and a cannabinoid could therefore reflect the net effect of appetite stimulating and appetite suppressing effects, not confounded by motor disruptive effects. Nevertheless, it is hypothesized that differences in receptor reserve, at least partly, underlie the differential sensitivity to SR141716A, as it can be expected that a potent behavioral effect, supposedly linked to a high degree of receptor reserve, will also require a high level of receptor occupation by an antagonist in order to be fully blocked.

Similarly, with respect to tolerance development, it is unclear whether this occurs to the same extent to each behavioral effect (for review, see Abood and Martin, 1992; Ameri, 1999). Consistent with other reports (e.g. Fan *et al.*, 1994), it was found in the present study that tolerance develops rapidly (i.e. within 4 days of twice-daily administration) to the hypothermic and analgesic effects of CP 55,940. However, when assessed under similar experimental conditions, it appeared that tolerance hardly develops to the response-rate suppressive effect of this compound. It is, however, possible that the apparent lack of tolerance development in the operant procedure (as compared with the hot-plate and body-temperature tests) is related to the fact that lower doses were used in the former test for the repeated administration experiment. It therefore remains to be tested whether repeated treatment with higher doses (dose range used in the hot-plate and body-temperature tests) induces a rightward shift of the dose–response curve of CP 55,940 in the operant procedure.

It is hypothesized that the differential sensitivity to tolerance development, as observed in the present experiment, also relates to the existence of different degrees of receptor reserve. According to that hypothesis, tolerance will not, or hardly, develop to those behavioral effects that are located to the left side of the cannabinoid dose-spectrum, and which are supposed to be linked to a relatively high degree of receptor reserve; whereas rapid tolerance will occur to those effects located at the right side of the dose-spectrum, linked to a lower degree of receptor reserve. Although the present study was not aimed at investigating the molecular mechanisms underlying differential tolerance development, it was reported that significant molecular changes in the brain cannabinoid system (such as receptor down-regulation and desensitization) were only observed after continuous exposure to relatively high doses of Δ^9-THC (rather in the dose range that induces hypothermic and analgesic effects; Breivogel *et al.*, 2003). However, Lamb *et al.* (2000) found that tolerance did occur to the response-rate suppressive effect of Δ^9-THC (Lamb *et al.*, 2000). This finding is possibly related to the suggestion made by Breivogel *et al.* (2003) that low-efficacy agonists (such as Δ^9-THC) induce a higher degree of receptor desensitization, than high-efficacy agonists (such as CP 55,940). Therefore, it remains to be investigated whether both type of compounds induce the same degree of tolerance

when different behavioral effects are assessed under the same experimental conditions.

Clinically established and promising potential therapeutic applications for cannabinoids include, amongst others, appetite-stimulant, anti-emetic, antispastic and neuroprotective effects (Felder and Glass, 1998; Mauler *et al.*, 2002). In addition, WIN 55,212-2 (Herzberg *et al.*, 1997), CP 55,940 (De Vry, 2002) and BAY 59-3074 (De Vry *et al.*, 2004c) were reported to produce antihyperalgesic and anti-allodynic effects, as assessed in the chronic constriction injury rat model of neuropathic pain (Bennett and Xie, 1988), suggesting that cannabinoids could be of particular benefit for the treatment of chronic neuropathic pain (for review, see Pertwee, 2001). As the efficacy of CP 55,940 in the latter model was assessed under the same experimental conditions as in the present study (route of administration, injection–test interval, vehicle, strain of rats), a clear assessment of the potency of the antihyperalgesic/anti-allodynic effects of this compound, relative to the other behavioral effects and side-effects, could be established. Indeed, as shown in Fig. 4 (left-hand panel), it appears that the antihyperalgesic/anti-allodynic effects of CP 55,940 occur at lower doses than those that induce antinociceptive effects (as assessed in the hot-plate test, a model representing nonpathological pain sensitivity) and hypothermic effects. This increased sensitivity to the antihyperalgesic/anti-allodynic effects of cannabinoids has been ascribed to the occurrence of a specific upregulation of cannabinoid CB_1 receptors in the thalamus under chronic pain conditions (Siegling *et al.*, 2001).

Taken together, it appears that the more the behavioral (or therapeutic) effect induced by a cannabinoid receptor agonist is situated at the left-hand side of the dose-spectrum, the more the effect is resistant to blockade by SR 141716A and to the development of tolerance. It should be noted, however, that this study was performed with exogenous cannabinoids and it remains to be determined whether these findings extend to endogenous cannabinoids such as anandamide. Interestingly, therapeutic effects of cannabinoids appear to occur at low to moderate doses (Felder and Glass, 1998; De Vry, 2002; Mauler *et al.*, 2002; De Vry *et al.*, 2004c), whereas the cannabinoid behavioral syndrome only becomes apparent at relatively high doses (Martin *et al.*, 1991; Compton *et al.*, 1992; Pertwee, 1999). As far as the cannabinoid behavioral syndrome represents an index of tolerability, this suggests that cannabinoids have a relatively favorable therapeutic/tolerability window. In addition, as tolerance appears to develop rapidly to the cannabinoid behavioral syndrome (e.g. present study; Fan *et al.*, 1994), but not to the potential therapeutic effects (Felder and Glass, 1998; De Vry *et al.*, 2004c), it is inferred that the therapeutic/tolerability window of cannabinoids will increase upon repeated administration. Clinical testing is eagerly awaited to further test this hypothesis and to confirm the broad therapeutic potential of cannabinoids predicted by preclinical studies.

References

Abood ME, Martin BR (1992). Neurobiology of marijuana abuse. *Trends Pharmacol Sci* **13**:201–206.

Ameri A (1999). The effects of cannabinoids on the brain. *Prog Neurobiol* **58**:315–348.

Balster RL, Prescott WR (1992). Δ^9-Tetrahydrocannabinol discrimination in rats as a model for cannabis intoxication. *Neurosci Biobehav Rev* **16**:55–62.

Bennett GI, Xie YK (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* **33**: 87–107.

Breivogel CS, Scates SM, Beletskaya IO, Lowery OB, Aceto MD, Martin BR (2003). The effects of Δ^9-tetrahydrocannabinol physical dependence on brain cannabinoid receptors. *Eur J Pharmacol* **459**:139–150.

Carriero D, Aberman J, Lin SY, Hill A, Makriyannis A, Salamone JD (1998). A detailed characterization of the effects of four cannabinoid agonists on operant lever pressing. *Psychopharmacology (Berl)* **137**:147–156.

Compton DR, Gold LH, Ward SJ, Balster RL, Martin BR (1992). Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from Δ^9-tetrahydrocannabinol. *J Pharmacol Exp Ther* **263**:1118–1126.

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

De Vry J, Jentzsch KR (2002). Discriminative stimulus effects of BAY 38-7271, a novel cannabinoid receptor agonist. *Eur J Pharmacol* **457**:147–152.

De Vry J, Jentzsch KR (2003). Intrinsic activity estimation of cannabinoid CB_1 receptor ligands in a drug discrimination paradigm. *Behav Pharmacol* **14**:471–476.

De Vry J, Schreiber R, Daschke A, Jentzsch KR (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.

De Vry J, Eckel G, Jentzsch KR (2004a). Partial agonist-like profile of the cannabinoid receptor antagonist SR141716A in a food-reinforced operant paradigm. *Behav Pharmacol* **15**:13–20.

De Vry J, Schreiber R, Eckel G, Jentzsch KR (2004b). Behavioral mechanisms underlying inhibition of food-maintained responding by the cannabinoid receptor antagonist/inverse agonist SR141716A. *Eur J Pharmacol* **483**: 55–63.

De Vry J, Denzer D, Reißmueller E, Eijckenboom M, Heil M, Meier H, Mauler F (2004c). 3-[2-Cyano-3-(trifluoromethyl)phenoxy]phenyl-4,4,4-trifluoro-1-butanesulfonate (BAY 59-3074): a novel cannabinoid receptor agonist with antihyperalgesic and anti-allodynic effects. *J Pharmacol Exp Ther* (in press).

Fan F, Compton DR, Ward S, Melvin L, Martin BR (1994). Development of cross-tolerance between Δ^9-tetrahydrocannabinol, CP 55,940 and WIN 55,212. *J Pharmacol Exp Ther* **271**:1383–1390.

Felder CC, Glass M (1998). Cannabinoid receptors and their endogenous agonists. *Annu Rev Pharmacol Toxicol* **38**:179–200.

Gaoni Y, Mechoulam R (1964). Isolation, structure and partial synthesis of an active constituent of hashish. *J Am Chem Soc* **86**:1646–1647.

Gifford AN, Bruneus M, Gatley SJ, Lan R, Makriyannis A, Volkow ND (1999). Large receptor reserve for cannabinoid actions in the central nervous system. *J Pharmacol Exp Ther* **288**:478–483.

Herzberg U, Eliav E, Bennett GJ, Kopin IJ (1997). The analgesic effects of *R*(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. *Neurosci Lett* **221**:157–160.

Järbe TUC, Hiltunen AJ, Mechoulam R (1989). Stereospecificity of the discriminative stimulus functions of the dimethylheptyl homologs of 11-hydroxy-Δ^8-tetrahydro-cannabinol in rats and pigeons. *J Pharmacol Exp Ther* **250**:1000–1005.

Järbe TUC, Lamb RJ, Liu Q, Makriyannis A (2003). (*R*)-Methanandamide and Δ^9-tetrahydrocannabinol-induced operant rate decreases in rats are not readily antagonized by SR-141716A. *Eur J Pharmacol* **466**:121–127.

Johnson MR, Melvin LS (1986.) The discovery of nonclassical cannabinoid analgesics. In: Mechoulam R (editor): *Cannabinoids as Therapeutic Agents*. Boca Raton: CRC Press; pp. 121–145.

Kirkham TC, Williams CM (2001). Endogenous cannabinoids and appetite. *Nutr Res Rev* **14**:65–86.

Lamb RJ, Järbe TUC, Makriyannis A, Lin S, Goutopoulos A (2000). Effects of Δ^9-tetrahydrocannabinol, (*R*)-methanandamide, SR 141716A, and D-amphetamine before and during daily Δ^9-tetrahydrocannabinol dosing. *Eur J Pharmacol* **398**:251–258.

Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, *et al.* (1991). Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. *Pharmacol Biochem Behav* **40**:471–478.

Mauler F, Mittendorf J, Horváth E, [8]De [8]Vry J (2002). Characterization of the diarylether sulfonylester (–)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271) as a potent cannabinoid receptor agonist with neuroprotective properties. *J Pharmacol Exp Ther* **302**:1–10.

Moldrich G, Wenger T (2000). Localization of the CB_1 cannabinoid receptor in rat brain. An immunohistochemical study. *Peptides* **21**:1735–1742.

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.

Pertwee RG (1999). Pharmacology of cannabinoid receptor ligands. *Cur Med Chem* **6**:635–664.

Pertwee RG (2001). Cannabinoid receptors and pain. *Prog Neurobiol* **63**: 569–611.

Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq MC (1998). Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of Δ^9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. *Life Sci* **63**:1–6.

Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, *et al.* (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* **350**:240–244.

Siegling A, Hofmann HA, Denzer D, Mauler F, De Vry J (2001). Cannabinoid CB_1 receptor upregulation in a rat model of chronic neuropathic pain. *Eur J Pharmacol* **415**:R5–R7.

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