Intrinsic activity estimation of cannabinoid CB_1 receptor ligands in a drug discrimination paradigm

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The present study estimated the apparent intrinsic activity of the cannabinoid CB_1 receptor ligands CP 55,940, Δ^9-tetrahydrocannabinol (Δ^9-THC) and SR 141716A in a highly sensitive *in vivo* assay. Rats were trained to discriminate the cannabinoid CB_1 receptor agonist CP 55,940 (either 0.03 or 0.014 mg/kg, i.p., $t=30$ min) from vehicle, in a two-lever food-reinforced procedure, and were subsequently tested with the three compounds. Although reduction of the training dose did not affect the maximum level of generalization or antagonism (>80% generalization for CP 55,940 and Δ^9-THC; 0% generalization and >80% antagonism for SR 141716A), the potency of the compounds was differentially affected. Thus, the generalization curves obtained with CP 55,940 and Δ^9-THC were shifted three- and sixfold to the left; whereas no potency difference was obtained for the antagonism of CP 55,940 by SR 141716A. The data are consistent with the hypothesis that the level of intrinsic activity of CP 55,940 is higher than that of Δ^9-THC, and that SR 141716A may have a very low level of intrinsic activity. It is concluded that variation of the training dose increases the sensitivity of the *in vivo* intrinsic activity estimation of cannabinoid CB_1 receptor ligands.
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Introduction

Classical cannabinoids, such as Δ^9-tetrahydrocannabinol (Δ^9-THC; the major active constituent of marijuana or *Cannabis sativa* L.; Gaoni and Mechoulam, 1964); as well as non-classical cannabinoids, such as CP 55,940 (Johnson and Melvin, 1986), WIN 55,212-2 (Compton *et al.*, 1992), and the recently discovered diarylether sulfonylester derivative, BAY 38-7271 (Mauler *et al.*, 2002), exert their effects through activation of cannabinoid CB_1 receptors (reviewed by Pertwee, 1997). The cannabinoid CB_1 receptor has been cloned (Matsuda *et al.*, 1990) and represents the cannabinoid receptor subtype, which is mainly expressed in the central nervous system (Herkenham *et al.*, 1990; Moldrich and Wenger, 2000). In general, the behavioral effects of cannabinoids can be attenuated by selective cannabinoid CB_1 receptor antagonists, such as SR 141716A (Rinaldi-Carmona *et al.*, 1994). This finding underscores the involvement of cannabinoid CB_1 receptors in the behavioral effects of cannabinoids.

Clinically established and potentially promising therapeutic applications for cannabinoid CB_1 receptor agonists include, amongst others, appetite stimulant, anti-emetic, antispastic, neuroprotective and analgesic effects; whereas cannabinoid CB_1 receptor antagonists suppress ingestive behavior and reduce body weight, and possibly have therapeutic potential for the treatment of drug addiction/alcoholism and schizophrenia (reviewed by Petitet and Imperato, 1998). Although cannabinoids may thus have many therapeutic uses, the optimal level of cannabinoid CB_1 receptor activation required for each indication remains to be determined. In addition, it is to be expected that liability for tolerance development and abuse will also be affected by the level of intrinsic receptor activity (e.g. Fan *et al.*, 1994; Tanda *et al.*, 2000). Development of behavioral assays that allow for a sensitive assessment of intrinsic activity at cannabinoid CB_1 receptors may therefore be helpful for the rational design of compounds with an optimal profile of activity. Despite the fact that CP 55,940 and Δ^9-THC are generally characterized *in vitro* as cannabinoid CB_1 receptor *full* agonist and *partial* agonist, respectively (Griffin *et al.*, 1998; Breivogel and Childers, 2000; Mauler *et al.*, 2002), they appear to behave rather similarly in various *in vivo* assays. Even in drug discrimination assays, which are generally considered to be highly sensitive behavioral paradigms, useful for assessment of drug–receptor interactions (e.g. Shannon and Holtzman, 1979; Colpaert *et al.*, 1980; De Vry and Slangen, 1986; Koek and Woods, 1989; Picker *et al.*, 1990), CP 55,940 and Δ^9-THC have been characterized as cannabinoid CB_1 receptor agonists, with no clear difference in level of intrinsic activity. Thus, in rats or monkeys trained to discriminate Δ^9-THC from vehicle, CP 55,940 was found to induce complete generalization (i.e. at least 80% generalization; Gold *et al.*, 1992); whereas rats trained to discriminate CP 55,940 from vehicle generalized completely to Δ^9-THC (Wiley *et al.*, 1995a; Mauler *et al.*,

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2002). On the other hand, SR 141716A, which behaves *in vitro* as a cannabinoid CB_1 receptor antagonist or inverse agonist (e.g. Rinaldi-Carmona *et al.*, 1994; Landsman *et al.*, 1997), also shows the profile of an antagonist in cannabinoid drug discrimination assays. Thus, pretreatment with SR 141716A appeared to antagonize the discriminative stimulus effects of diverse cannabinoid CB_1 receptor agonists, including Δ^9-THC, CP 55,940, WIN 55,212-2 and BAY 38-7271 (Wiley *et al.*, 1995a, b; Mansbach *et al.*, 1996; Pério *et al.*, 1996; Järbe *et al.*, 2001; De Vry and Jentzsch, 2002; Mauler *et al.*, 2002). In these studies, doses of 0.3 to 1 mg/kg of SR 141716A were generally sufficient to reduce drug-appropriate responding to 20% or less. In addition, it was reported that these doses of SR 141716A induced a dose-dependent rightward shift of the dose–response (generalization) curve obtained with Δ^9-THC, in rats trained to discriminate the latter compound from vehicle (Järbe *et al.*, 2001). When tested alone, SR 141716A typically induced not more than 20% generalization (even if tested up to 10 mg/kg). These findings are therefore compatible with its characterization as a cannabinoid CB_1 receptor antagonist. However, although the minor degree of generalization (i.e. around 17–20% generalization) obtained by De Vry and Jentzsch (2002) and Mauler *et al.* (2002) would generally be considered as non-significant, it cannot be excluded that this reflects the existence of a low degree of intrinsic activity of SR 141716A at those cannabinoid CB_1 receptors involved in the discriminative effects of cannabinoids. Indeed, it is noteworthy that in pigeons trained to discriminate Δ^9-THC from vehicle, high doses of SR 141716A were reported to induce partial generalization (i.e. 57% and 43% generalization at 10 and 17 mg/kg, respectively; Mansbach *et al.*, 1996). Although the phenomenon of partial generalization can tentatively be explained by various mechanisms (for discussion, see Koek and Woods, 1989), these findings open the possibility that SR 141716A behaves, particularly in *in vivo* assays, as a cannabinoid CB_1 receptor *partial* agonist (or low intrinsic activity agonist), rather than as a silent cannabinoid CB_1 receptor antagonist.

Variation of the training dose in a drug discrimination assay has previously been used successfully to differentiate partial agonists from silent antagonists and full agonists (e.g. Shannon and Holtzman, 1979; Colpaert *et al.*, 1980; De Vry and Slangen, 1986; Koek and Woods, 1989; Picker *et al.*, 1990). Also, in the case of cannabinoids (i.e. Δ^9-THC), it was demonstrated that training dose variation was instrumental to detect differences in intrinsic activity between Δ^9-THC and (*R*)-methanandamide (Järbe *et al.*, 1998). The present study, therefore, investigated whether this manipulation, in rats trained to discriminate CP 55,940 from vehicle, could reveal partial agonist properties of SR 141716A, as well as differences in the intrinsic activity of CP 55,940 and Δ^9-THC. A preliminary account of this study was presented at the Eighth EBPS Meeting, Boston (De Vry and Jentzsch, 1999).

Methods

Subjects

Male Wistar rats were purchased from Harlan–Winkelmann (HsdCpb: WU, Borchen, Germany). Body weight upon arrival at the laboratory was around 250 g, which gradually increased up to about 500 g during the course of the study. Rats were housed individually in Makrolon® type 3 cages under a normal 12-hour light period (light on at 07.00 hours). The animals had restricted access to food (approximately 13 g/day, standard pellets, Ssniff Spezialdiäten GmbH, Soest, Germany) and had free access to water. Room temperature was maintained at 20–22°C. The rats had been used in a previous study in which they had successfully learned to discriminate CP 55,940 (0.03 mg/kg, i.p., $t-30$ min) from vehicle and in which they were tested subsequently with a number of cannabinoid receptor ligands (Mauler *et al.*, 2002). The procedure followed the guidelines for the use of animals, as given by the German government, and was approved by the local authorities (Regierungspräsidium Düsseldorf, Germany).

Apparatus

Experiments were performed in sound- and light-attenuated standard operant chambers (Coulborn Instruments, Lehigh Valley, Pensylvania, USA). The chambers were equipped with two levers equidistant from a food tray between the levers. Food reinforcement (45 mg precision pellets, Bio-Serv, NJ, USA) was delivered by an automated food dispenser located outside 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 12.00 hours.

Drug discrimination procedure

In general, the procedure described by De Vry and Jentzsch (2002) and Mauler *et al.* (2002) was followed. After initial shaping to lever press for food reinforcement, the rats ($n = 16$) were trained to discriminate 0.03 mg/kg CP 55,940 (i.p., $t-30$ min) from vehicle in a standard two-lever, fixed-ratio 10 operant procedure. Daily sessions were conducted, which were terminated after either 50 reinforcers or after 10 min, whichever came first. For half of the animals, responses on the left lever were reinforced after CP 55,940, for the other half, responses on this lever were reinforced after vehicle. The rats were injected with drug or vehicle according to the following sequence: D–D–V–D–V//V–D–V–V–D//D–V–D–V–V//D–D–V–D–V (D = drug, V = vehicle, // = no sessions during the weekends) with repetition. The discrimination

criterion consisted of 10 consecutive sessions in which no more than nine responses occurred on the non-reinforced lever before the first reinforcer was obtained. After reaching discrimination criterion, the rats were given generalization and antagonism test sessions. Test sessions were performed when the number of incorrect responses was not more than four on three consecutive training sessions, and when at least 20 reinforcers were obtained per session. During test sessions, responding on the selected lever, i.e. the lever on which 10 responses accumulated first, was reinforced for the remainder of the session. Generalization and antagonism tests were separated by at least three training sessions in which vehicle and drug were correctly discriminated, i.e. fewer than five incorrect responses prior to the first reinforcer. Rats were tested with different doses of CP 55,940, as well as with a number of cannabinoid receptor ligands (including Δ^9-THC and SR 141716A; Mauler *et al.*, 2002). Subsequently, one subgroup was retrained to discriminate 0.014 mg/kg CP 55,940 (i.p., $t-30$ min, $n=6$, randomly selected from the initial 16 rats); whereas the other subgroup ($n=10$) was maintained on the initial training dose. When the former group had reached discrimination criterion (under the 0.014 mg/kg training dose condition), both groups were submitted again to generalization and antagonism tests. The animals were tested with different doses of CP 55,940 (0, 0.0014, 0.0044 and 0.014 mg/kg in the 0.014 mg/kg training dose group; 0, 0.003, 0.01 and 0.03 mg/kg in the 0.03 mg/kg training dose group; i.p., $t-30$ min) and Δ^9-THC (0, 0.03, 0.1, 0.3 and 1 mg/kg in the 0.014 mg/kg training dose group; 0, 0.3, 1 and 3 mg/kg in the 0.03 mg/kg training dose group; i.p., $t-30$ min). In the antagonism test sessions, different doses of SR 141716A (0, 0.1, 0.3, 1 and 3 mg/kg, i.p.) were injected 10 min before administration of the training dose of CP 55,940 (0.014 and 0.03 mg/kg, respectively), and 30 min after the latter administration rats were tested. Finally, both groups were also tested with SR 141716A (0, 0.3, 1, 3 and 10 mg/kg) in combination with vehicle, according to the same application schedule as in the antagonism test sessions.

Drugs

CP 55,940 {(−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexanol} and SR 141716A [*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 AG (Wuppertal, Germany). Δ^9-tetrahydrocannabinol was obtained from Sigma–Aldrich Chemie (Steinheim, Germany). All compounds were dissolved in 2.5% Solutol® HS 15 (12-hydroxystearic-acid ethoxylate, BASF AG, Ludwigshafen, Germany), 2.5% ethanol (ethanol absolute, 99.8%, Riedel-de Haën, Seelze, Germany) and distilled water or 0.9% NaCl (saline). Injection volume was 2 ml/kg body weight.

Data analysis

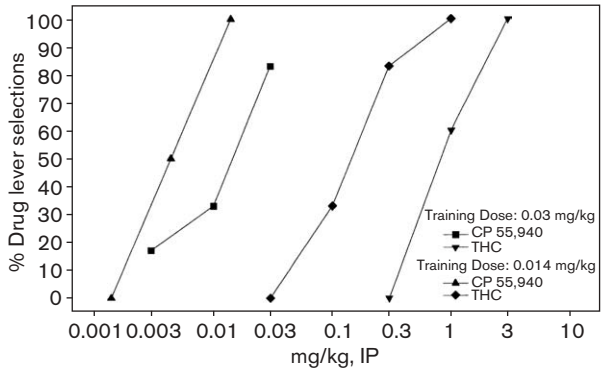
Generalization and antagonism test results were expressed as the percentage of rats that selected the drug lever (% drug lever selections). Generalization was considered to be complete if at least 80% drug lever selections was obtained, whereas antagonism was considered to be complete if 20%, or fewer, drug-lever selections was obtained. The response rate (i.e. the number of responses/s) obtained during the test sessions was analyzed, for each compound separately, by ANOVA, followed by Tukey's *post-hoc* comparisons. In addition, the percentage of animals that selected a lever (either drug or vehicle lever) was determined as an index of behavioral disruption (% lever selections). Least-square linear regression analysis was used to estimate ED_{50} and ID_{50} values, and their 95% confidence limits, after log-probit conversion of the data. ED_{50} and ID_{50} values with non-overlapping 95% confidence limits were considered to be significantly different.

Results

All 16 rats learned to discriminate CP 55,940 (0.03 mg/kg, i.p.) from vehicle, the median number of sessions to reach criterion being 38 (range: 29–103 sessions; Mauler *et al.*, 2002). Retraining of one subgroup on the 0.014 mg/kg dose of CP 55,940 required a median number of 31 (range: 10–60 sessions) additional sessions. As shown in Fig. 1, generalization tests with CP 55,940 resulted in dose-dependent and complete generalization in both groups; the ED_{50} values (95% confidence limits) being: 0.004 (0.003–0.008) and 0.012 (0.004–0.030) mg/kg, in the 0.014 and 0.03 mg/kg training dose group, respectively. Dose-dependent and complete generalization was also obtained after administration of Δ^9-THC [ED_{50} values (95% confidence limits): 0.16 (0.08–0.31) and 0.92 (0.46–1.84) mg/kg, in the 0.014 and 0.03 mg/kg training dose group, respectively]. Generalization occurred in the absence of behavioral disruption. Thus, all rats selected a lever at each dose tested and response rate was not affected by either compound. In the 0.014 mg/kg training dose group, mean response rate after testing the different doses of CP 55,940 (including vehicle) ranged from 0.77 to 0.83 responses/s [$F(3,28)=1.03$, $P>0.05$], and after Δ^9-THC ranged from 0.74 to 0.81 responses/s [$F(4,23)=0.42$, $P>0.05$]; whereas, in the 0.03 mg/kg training dose group, mean response rate ranged from 0.75 to 0.83 responses/s [$F(3,20)=1.42$, $P>0.05$] after testing of CP 55,940, and ranged from 0.82 to 0.83 responses/s [$F(3,12)=0.56$, $P>0.05$] after Δ^9-THC.

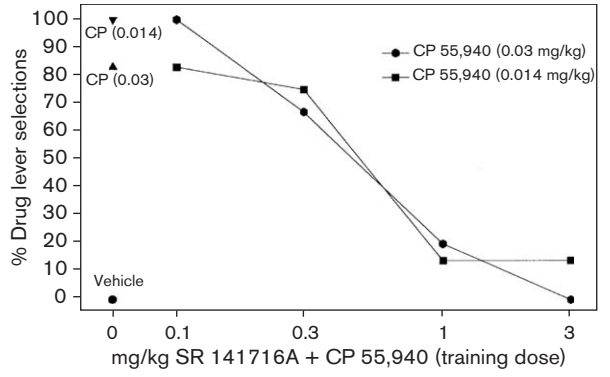
Pretreatment with SR 141716A resulted in dose-dependent and complete antagonism of CP 55,940 in both groups (Fig. 2). Antagonism by SR 141716A was equipotent in both training dose groups [ID_{50} values (95% confidence limits): 0.45 (0.16–1.26) and 0.50 (0.25–1.01) mg/kg, in the 0.014 and 0.03 mg/kg training

Fig. 1



Effect of training dose variation on the generalization of CP 55,940 and Δ^9-tetrahydrocannabinol (Δ^9-THC) in rats trained to discriminate CP 55,940 from vehicle (i.p., $t-30$ min) in a standard two-lever food-reinforced procedure. The training dose of CP 55,940 was either 0.03 mg/kg ($n=6$) or 0.014 mg/kg ($n=10$). Vehicle administration resulted in 0% drug-lever selections in both groups.

Fig. 2



Effect of training dose variation on the antagonism of CP 55,940 (CP, training dose) by SR 141716A (i.p., $t-40$ min) in rats trained to discriminate CP 55,940 from vehicle (i.p., $t-30$ min) in a standard two-lever food-reinforced procedure. The training dose of CP 55,940 was either 0.03 mg/kg ($n=6$) or 0.014 mg/kg ($n=10$).

dose group, respectively]. Antagonism occurred in the absence of behavioral disruption. Thus, all rats selected a lever at each test dose combination and response rate was not affected by SR 141716A in either training dose group [mean response rate range in the 0.014 mg/kg and 0.03 mg/kg training dose group: 0.67–0.83 responses/s, $F(4,31) = 2.22$, $P > 0.05$, and 0.54–0.83 responses/s, $F(4,22) = 2.79$, $P > 0.05$, respectively]. SR 141716A (combined with vehicle) did not induce generalization (0% drug lever selections, at each dose tested). However, response rate was significantly affected by SR 141716A in both training groups [0.014 mg/kg training dose group: $F(4,31) = 17.64$, $P < 0.001$; 0.03 mg/kg training dose group: $F(4,23) = 7.42$, $P < 0.001$], and the reduction in response rate was statistically significant at 10 mg/kg. Thus, in the 0.014 mg/kg training dose group, response rate declined from 0.77 responses/s (vehicle/vehicle) to 0.13 responses/s (10 mg/kg, $P < 0.001$), and in the 0.03 mg/kg training dose group, response rate declined from 0.83 responses/s (vehicle/vehicle) to 0.20 responses/s (10 mg/kg, $P < 0.001$). Behavioral disruption was also reflected by an increase in the percentage of rats not selecting a lever. Thus, in the 0.014 mg/kg training dose group, this percentage was 17% and 50%, at 3 and 10 mg/kg, respectively, and in the 0.03 mg/kg training dose group, it was 17% at 10 mg/kg.

Discussion

It has been previously demonstrated that the discriminative stimulus induced by a cannabinoid CB_1 receptor agonist is pharmacologically specific and a sensitive *in vivo* correlate of cannabinoid CB_1 receptor activation (for reviews, see Balster and Prescott, 1992; Barrett *et al.*, 1995). Also, in the case of the cannabinoid CB_1 receptor *full* agonist CP 55,940, it can be argued that the cue is mediated by activation of cannabinoid CB_1 receptors, as it was found that: (1) the order of potency of generalization to cannabinoid CB_1 receptor agonists from diverse chemical classes closely correlates with their affinity at cannabinoid CB_1 receptors; and (2) the CP 55,940 cue can be antagonized by pretreatment with the selective cannabinoid CB_1 receptor antagonist, SR 141716A (this study; Wiley *et al.*, 1995a; De Vry and Jentzsch, 2002; Mauler *et al.*, 2002). Moreover, it is very likely that the cannabinoid cue is centrally mediated, as it was found that intracerebral administration of Δ^9-THC induced drug-appropriate responding in rats trained to discriminate this compound from vehicle after systemic administration (Kallman *et al.*, 1984), and pretreatment with a peripherally acting cannabinoid CB_1 receptor antagonist failed to block the discriminative effects of WIN 55,212-2, a cannabinoid CB_1 receptor agonist (Pério *et al.*, 1996).

The present finding that rats, initially trained to discriminate a relatively low dose (0.03 mg/kg) of CP 55,940 from vehicle, are able to learn to discriminate a lower dose (0.014 mg/kg), is in accordance with previous studies in which a similar dose-fading procedure was used for discrimination learning of low doses of Δ^9-THC (Järbe *et al.*, 1998). The observation that the ED_{50} value of generalization to CP 55,940 is relatively proportional to the training dose (i.e. a reduction of 50% in the training dose level leads to a threefold shift of the dose–response curve) is also consistent with previous studies performed with Δ^9-THC or compounds from other pharmacological classes (e.g. Shannon and Holtzman, 1979; Colpaert *et al.*, 1980; De Vry and Slangen, 1986; Koek and Woods, 1989; Picker *et al.*, 1990). The latter studies demonstrated that cross-generalization, to a compound with a similar level of intrinsic receptor activity as the training drug, also

resulted in a similar shift of the generalization curve as that obtained with the training drug, after reduction of the training dose. On the other hand, these studies showed that cross-generalization, to a compound with a lower level of intrinsic activity, led to an overproportional shift of the generalization curve to the left. In the present study, training dose reduction resulted in a shift of the generalization curve obtained with Δ^9-THC, which was larger than the shift obtained with CP 55,940 (i.e. sixfold, instead of threefold). This finding suggests that the intrinsic activity of CP 55,940 at cannabinoid CB_1 receptors is higher than that of Δ^9-THC, and is compatible with the *in vitro* characterization of these compounds as *full* and *partial* agonist, respectively (Griffin *et al.*, 1998; Breivogel and Childers, 2000; Mauler *et al.*, 2002).

The finding that pretreatment with SR 141716A resulted in complete (i.e. less than 20% drug-appropriate responding) antagonism of the CP 55,940 cue is consistent with previous studies in which it was reported that SR 141716A was able to block the cue induced by various cannabinoid CB_1 receptor agonists (Mansbach *et al.*, 1996; Pério *et al.*, 1996; Järbe *et al.*, 2001; De Vry and Jetzsch, 2002; Mauler *et al.*, 2002; Wiley *et al.*, 1995a, b). Interestingly, however, training dose reduction did not result in a dose-proportional leftward shift of the antagonist dose–response (ID_{50} values obtained with SR 141716A were virtually identical in both training groups). Because it was previously demonstrated that training dose reduction results in dose-proportional leftward shifts of the antagonist curve in the case of a (silent) receptor antagonist, but to less pronounced, or even no shift, in the case of a partial agonist (e.g. Shannon and Holtzman, 1979; Colpaert *et al.*, 1980; De Vry and Slangen, 1986; Koek and Woods, 1989; Picker *et al.*, 1990), the apparent absence of a leftward shift of the SR 141716A dose–response curve is not compatible with its characterization as a silent receptor antagonist. This finding could indicate that SR 141716A has some intrinsic activity at those cannabinoid CB_1 receptors mediating the CP 55,940 cue. On the other hand, however, testing of SR 141716A alone did not result in a higher level of generalization after training dose reduction (which would be expected in the case of a partial agonist). Therefore, it is very likely that, if the compound has indeed some cannabinoid CB_1 receptor agonist activity, the level of intrinsic activity is probably very low. Interestingly, SR 141716A dose-dependent reduced response rate, a finding that was also reported previously in similar food-reinforced operant procedures (e.g. Freedland *et al.*, 2000; Järbe *et al.*, 2001; Mauler *et al.*, 2002). In addition, response rate-decreasing effects induced by SR 141716A in a food-reinforced operant paradigm could not, or only partially, be reversed by pre- or post-treatment with cannabinoid CB_1 receptor agonists (De Vry and Jentzsch, unpublished; Järbe *et al.*, 2003). These findings, therefore, could also indicate that SR 141716A has cannabinoid CB_1 receptor partial agonist properties, or alternatively, may reflect an additional, unidentified mechanism of action (for discussion, see Järbe *et al.*, 2003). Finally, it should be mentioned that further work is needed to exclude the possibility that the generalization and antagonism test results of the present study were confounded by using a training technique based on dose-fading. With respect to this issue it should be noted, however, that previous studies comparing this technique with a paradigm in which the subjects were trained on different doses from the onset of discrimination training, did not result in essentially different test results (e.g. De Vry and Slangen, 1986; Järbe *et al.*, 2000, 2001).

Although classical and non-classical cannabinoids show differences in apparent intrinsic activity at cannabinoid CB_1 receptors as assessed *in vitro* (Griffin *et al.*, 1998; Breivogel and Childers, 2000; Mauler *et al.*, 2002), they generally induce a qualitatively similar profile of behavioral activity across a very broad dose-spectrum. Thus, as assessed in rats, discriminative effects are obtained at the left side of the dose-spectrum, followed, at higher doses, by suppressant effects on operant behavior, hypothermia and sedation and, finally, at the right side of the dose-spectrum, by catalepsy (Martin *et al.*, 1991; De Vry, 2002; Mauler *et al.*, 2002). Because the major difference in behavioral activity between these compounds relates to potency, rather than efficacy, it has been hypothesized that the behavioral profile of cannabinoids is mainly determined by the compound's receptor affinity, rather than its intrinsic activity at these receptors (De Vry, 2002). At the molecular level, this phenomenon could be explained by the presence of a high degree of receptor reserve/receptor–effector coupling (Gifford *et al.*, 1999; Breivogel and Childers, 2000;). According to this hypothesis, it can be postulated that, once the intrinsic activity of a cannabinoid surpasses a rather low, but critical, level of intrinsic activity, the compound will be able to produce a typical cannabinoid-like behavioral profile. In addition, the existence of a high degree of receptor reserve/receptor–effector coupling may also be responsible for the finding that behavioral effects induced by these ligands are relatively resistant to treatment with antisense oligodeoxynucleotides complementary to cannabinoid CB_1 receptor mRNA (De Vry *et al.*, 1999). It should be noted, however, that such behavioral observations were obtained after acute administration, and it is conceivable that different levels of intrinsic activity may result in different levels of behavioral efficacy when the compounds are administered repeatedly (as is the case under therapeutic treatment conditions). Indeed, it appears that the more the behavioral (or therapeutic) effect induced by a cannabinoid is situated at the left side of the dose-spectrum, the more the effect is resistant to the development of tolerance, as well as to blockade by SR 141716A (De Vry, 2002). Again, this phenomenon can be

explained tentatively by the existence of different degrees of receptor reserve (spare receptors) and/or receptor–effector coupling. According to this hypothesis, the most potent behavioral effects are mediated by receptor populations with a relatively high degree of receptor reserve/receptor–effector coupling; whereas the least potent effects are mediated by receptor populations with a relatively low degree of receptor reserve/receptor–effector coupling. From this hypothesis it can be predicted that the therapeutic window of cannabinoid receptor agonists will increase upon repeated administration, as tolerance will rapidly develop to the side-effects (which are generally obtained at relatively high doses), but hardly, or not, to the therapeutic effects (which are generally obtained at relatively low doses). Clearly, extensive research will be needed to assess in more detail the optimal level of intrinsic receptor activity required for each potential therapeutic application of a cannabinoid; especially in the light of chronic application and the relationship between intrinsic receptor activity and liability for tolerance development and abuse (e.g. Fan *et al.*, 1994; Tanda *et al.*, 2000). Clinical testing is eagerly awaited to test these hypotheses and to confirm the broad therapeutic potential of cannabinoids predicted by preclinical studies.

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