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Discriminative stimulus effects of BAY 38-7271, a novel cannabinoid receptor agonist

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Abstract

BAY 38-7271 [(−)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate] is a novel, highly potent and selective cannabinoid CB_1/CB_2 receptor agonist with neuroprotective properties. It was the aim of the present study to further confirm its cannabinoid CB_1 receptor agonist properties in a highly sensitive in vivo assay. Male Wistar rats ($n=24$) were trained to discriminate BAY 38-7271 (0.05 mg/kg, i.p., $t-30$ min) from vehicle in a fixed-ratio:10, food-reinforced two-lever standard procedure. The animals acquired the discrimination after a median number of 52 training sessions. BAY 38-7271 generalized dose-dependently when tested after different routes of administration (ED_{50}: 0.018 mg/kg, i.p.; 0.001 μg/kg, i.v.; 0.18 mg/kg, p.o.). A time-dependency study indicated that the cue (0.05 mg/kg, i.p.) was detectable between 15 min and 4 h, with a maximum of generalization obtained at 30 min after administration. Pretreatment with the selective cannabinoid CB_1 receptor antagonist SR 141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride] completely antagonized the effects of BAY 38-7271 (ID_{50}: 1.1 mg/kg, i.p.). Dose-dependent and complete generalization was also obtained after i.p. administration of the reference cannabinoid CB_1 receptor agonists HU-210 [(−)-11-OH-Δ^8-tetrahydrocannabinol-dimethylheptyl, ED_{50}: 0.003 mg/kg], CP 55,940 {(−)-*cis*-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol, 0.007 mg/kg}, WIN 55,212-2 [(*R*)-4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphtalenylcarbonyl)-6*H*-pyrrolo [3,2,1-*ij*] quinolin-6-one, 0.28 mg/kg] and (−)-Δ^9-tetrahydrocannabinol (0.34 mg/kg). The present study confirms that BAY 38-7271 is a highly potent cannabinoid CB_1 receptor agonist in vivo.

Keywords: Animal model; Cannabinoid CB_1 receptor; Drug discrimination; Neuroprotectant; (Rat)

1. Introduction

BAY 38-7271 [(−)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate] is a structurally novel cannabinoid CB_1/CB_2 receptor agonist with neuroprotective properties in rat models of traumatic brain injury and cerebral ischemia (Mauler et al., 2002). In vitro, BAY 38-7271 was characterized as a highly potent cannabinoid CB_1 receptor ligand ($K_i=0.46-1.85$ nM, as assessed at rat brain or human cortex membranes, or at human cannabinoid CB_1 receptors), with full agonist properties at this receptor (as indicated by [^{35}S]GTPγS binding assays using rat or human cannabinoid CB_1 receptors). Besides additional affinity to cannabinoid CB_2 receptors ($K_i=6$ nM, recombinant human cannabinoid CB_2 receptor), the range between specific cannabinoid receptor binding and any interaction with other receptors, channels or enzymes was at least two orders of magnitude, suggesting that BAY 38-7271 is a selective cannabinoid receptor agonist.

Behavioral studies have suggested that the compound is also a potent cannabinoid CB_1 receptor agonist in vivo (Mauler et al., 2002). Thus, in rats trained to discriminate the reference cannabinoid CB_1 receptor agonist {(−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol} (CP 55,940, Johnson and Melvin, 1986) from vehicle, BAY 38-7271 was found to induce complete generalization after systemic administration (ED_{50}: 0.015 mg/kg, i.p.). As it has been proposed that the discriminative stimulus effects of CP 55,940 are mediated by activation of cannabinoid CB_1 receptors (Wiley et al., 1995b), this result supports the notion that BAY 38-7271 is a potent cannabinoid CB_1 receptor agonist in vivo. In addition, BAY 38-7271 was found to induce a reduction in body temperature at higher doses (i.e., ≥0.3 mg/kg, i.p., Mauler et al., 2002). As such hypothermic effects have also

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been described for various, structurally diverse, cannabinoid CB_1 receptor agonists (Martin et al., 1991; Pertwee, 1984), this finding is again compatible with its characterization as a cannabinoid CB_1 receptor agonist. Moreover, it was found in both in vivo models that the effects of BAY 38-7271 and CP 55,940 were completely blocked by pretreatment with the selective cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR 141716A; Rinaldi-Carmona et al., 1994).

The present study was performed to further characterize the discriminative stimulus effects of BAY 38-7271, with the aim to confirm its cannabinoid CB_1 receptor agonist properties in a highly sensitive in vivo assay. Rats were trained to discriminate a relatively low dose of the compound (0.05 mg/kg, i.p.) from vehicle in a standard two-lever food-reinforced drug discrimination procedure, and subsequently the effects of BAY 38-7271 were compared with those of the reference cannabinoid CB_1 receptor agonists CP 55,940, HU-210 [(−)-11-OH-Δ^8-tetrahydrocannabinol-dimethylheptyl; Järbe et al., 1989], WIN 55212-2 [(*R*)-4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphtalenylcarbonyl)-6*H*-pyrrolo [3,2,1-*ij*]quinolin-6-one; Compton et al., 1992] and (−)-Δ^9-tetrahydrocannabinol (Gaoni and Mechoulam, 1964) in generalization test sessions. In order to further confirm the involvement of cannabinoid CB_1 receptors, it was tested whether pretreatment with the cannabinoid CB_1 receptor antagonist SR 141716A was able to block the discriminative stimulus effects of BAY 38-7271.

2. Material and methods

2.1. Animals

Male Wistar rats were purchased from Harlan-Winkelmann (HsdCpb: WU, Borchen, Germany). Body weight upon arrival at the laboratory was around 160 g, which gradually increased up to about 500 g during the course of the drug discrimination study. Rats were individually housed in Makrolon® type 3 cages under a normal 12 h light period (light on at 7:00 a.m.). The animals had restricted access to food (approximately 13 g/day, standard pellets, Ssniff Spezialdiäten, Soest, Germany) and were offered water ad libitum. Room temperature was maintained at 20–22 °C. The procedure followed the guidelines for the use of animals, as given by the German government, and was approved by the local authorities (Regierungspresidium Dusseldorf, Germany).

2.2. Apparatus

Sessions were performed in sound- and light-attenuated standard operant chambers (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, NJ, 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 9:00 and 12:00 a.m.

2.3. Procedure

In general, the procedure described by De Vry and Jentzsch (1998) and Mauler et al. (2002) was followed. After initial shaping to lever press for food reinforcement, the rats ($n = 24$) were trained to discriminate BAY 38-7271(0.05 mg/kg, 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 BAY 38-7271, 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. 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. Test sessions were performed when this number of incorrect responding 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., less than five incorrect responses prior to the first reinforcer. The animals were tested with different doses of BAY 38-7271 (0 and 0.006–0.1 mg/kg, i.p., $t - 30$ min; 0 and 0.001–0.003 mg/kg, i.v., $t - 30$ min; 0 and 0.1–1 mg/kg, p.o., $t - 1$ h). In the time-dependency studies, BAY 38-7271 was tested 0.25, 0.5, 1, 2, 4 and 8 h following i.p. administration of 0.05 mg/kg, and 0.5, 1, 2 and 4 h following p.o. administration of 1 mg/kg, during different test sessions. In the antagonism study, pretreatment with SR 141716A (0 and 0.3–3 mg/kg, i.p.) occurred 10 min before treatment with BAY 38-7271 (0.05 mg/kg or vehicle, i.p., $t - 30$ min). Further generalization tests were performed with the reference cannabinoid CB_1 receptor agonists HU-210 (0.001–0.03 mg/kg, i.p.), CP 55,940 (0.003–0.03 mg/kg, i.p.; 0.01–0.1 mg/kg, p.o.), WIN 55,212-2 (0.1–3 mg/kg) and (−)-Δ^9-tetrahydrocannabinol (0.1–3 mg/kg, i.p.; 0.1–3 mg/kg, p.o.). In general,

each dose of a test compound or test compound combination was tested in six rats, randomly allocated to each test condition (except for the generalization tests with the training drug after i.p. administration, which were performed in 11–16 rats). All compounds were tested 30 min after administration (except for HU-210, which was tested after 2 h).

2.4. Drugs

BAY 38-7271 [(−)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate], SR 141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride], HU-210 [(−)-11-OH-Δ^8-tetrahydrocannabinol-dimethylheptyl], and CP 55,940 {(−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol} were synthesized by the Chemistry Department of Bayer (Wuppertal, Germany). (−)-Δ^9-Tetrahydrocannabinol was obtained from Sigma-Aldrich (Steinheim, Germany) and WIN 55212-2 [(*R*)-4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphtalenylcarbonyl)-6*H*-pyrrolo[3,2,1-*ij*]quinolin-6-one] was obtained from RBI (Natick, MA, USA). All compounds were dissolved in 2.5% Solutol® HS 15 (12-hydroxystearic-acid ethoxilate, BASF, Ludwigshafen, Germany), 2.5% ethanol (ethanol absolute, 99.8%, Riedel-de Haen, Sellze, Germany) and distilled water or 0.9% NaCl (saline). Application volume was 2 ml/kg body weight.

2.5. Data analysis

Test results were expressed as the percentage of rats that selected the drug lever (% Drug Lever Selections). In addition, the percentage of animals that selected a lever (either drug or vehicle lever) was determined as an index of behavioral disruption (i.e., % Lever Selections). Least-square linear regression analysis was used to estimate ED_{50}, ID_{50} and $T_{1/2}$ values (and their 95% confidence limits) after log-probit conversion of the data. Generalization was considered to be complete if at least 80% drug lever selections was obtained, whereas antagonism was considered to be complete if less than 20% drug lever selections was obtained. In order to compare the generalization data obtained with the various cannabinoid CB_1 receptor agonists in the present BAY 38-7271 drug discrimination with those obtained previously in a CP 55,940 (0.03 mg/kg, i.p.) drug discrimination (using identical training and test conditions; Mauler et al., 2002), a linear regression analysis was performed on the respective $\log_{10}$-transformed ED_{50} values. In order to correlate in vivo activity of these compounds (BAY 38-7271 drug discrimination) with in vitro actvity (cannabinoid CB_1 receptor binding using rat brain membranes and [^{3}H]BAY 38-7271 as radiolabeled ligand; Mauler et al., 2002), a regression analysis was performed on the respective $\log_{10}$-transformed ED_{50} values and $\log_{10}$-transformed K_i values.

3. Results

All 24 rats learned to discriminate BAY 38-7271 (0.05 mg/kg, i.p.) from vehicle, the median number of sessions to reach criterion being 52 (range: 26–78 sessions). The generalization obtained with BAY 38-7271 was dose-dependent (Fig. 1, ED_{50} value in Table 1, complete generalization at 0.05 mg/kg) and occurred in the absence of behavioral disruption (100% Lever Selections at each dose), except for the highest dose tested (0.1 mg/kg) at which 2 out of 11 rats did not select a lever. Also after i.v. and p.o. administration, BAY 38-7271 induced dose-dependent generalization (data not shown, complete generalization obtained at 0.003 mg/kg, i.v. and 1 mg/kg, p.o., ED_{50} values in Table 1).

A time-dependency study indicated that the discriminative effects of 0.05 mg/kg BAY 38-7271 reached maximal intensity at 30 min after i.p. administration, and disappeared within 8 h [$T_{1/2}$ (95% confidence limits): 109 (64–186) min, 100% Lever Selections at each dose, Fig. 2]. After p.o. administration of 1 mg/kg BAY 38-7271, the cue was detectable between 30 min and 4 h and was maximal after 1 h [100% generalization, $T_{1/2}$ (95% confidence limits): 181 (126–261) min, data not shown].

Pretreatment with the selective cannabinoid CB_1 receptor antagonist SR 141716A dose-dependently blocked the discriminative effects of BAY 38-7271 (Fig. 3, ID_{50} value in Table 1), with complete antagonism obtained at 3 mg/kg. Testing of the antagonist alone (in combination with vehicle) did not induce more than 20% Drug Lever Selections (0%, 17% and 20% generalization after 0.3, 1 and 3 mg/kg, respectively). All rats selected a lever, except at the highest dose tested of SR 141716A (3 mg/kg) at which one out of six rats tested failed to select a lever, both after combination with vehicle and after combination with BAY 38-7271.

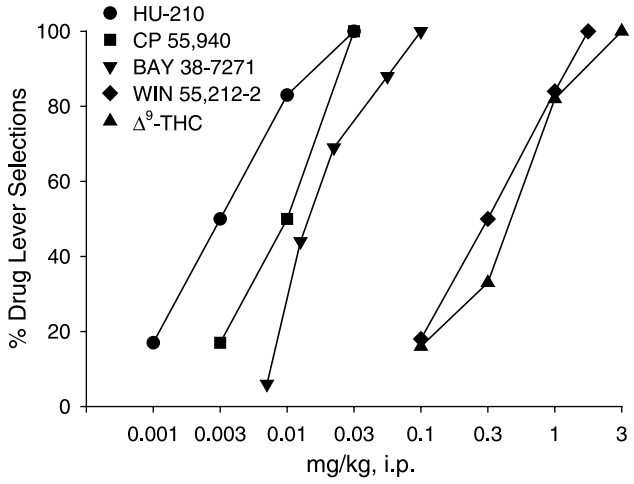

Fig. 1. Dose-dependent generalization induced by cannabinoid CB_1 receptor agonists in rats trained to discriminate BAY 38-7271 (0.05 mg/kg) from vehicle. Compounds were administered i.p., 0.5 h before test, except for HU-210 which was administered 2 h before test. Δ^9-THC=(−)-Δ^9-tetrahydrocannabinol. $n=5–6$ per dose (except for BAY 38-7271, $n=11–16$).

Table 1
Summary of test results with cannabinoid CB_1 receptor ligands in rats trained to discriminate BAY 38-7271 from vehicle

Compound	Application	Type of test	% Drug lever selections ED_{50} (95% CL)[a]
BAY 38-7271	i.p.	generalization	0.018 (0.012–0.026)
	i.v.	generalization	0.001 (NC)
	p.o.	generalization	0.18 (0.10–0.35)
HU-210	i.p.	generalization	0.003 (0.001–0.010)
CP 55,940	i.p.	generalization	0.007 (0.003–0.015)
	p.o.	generalization	0.023 (0.010–0.056)
WIN 55,212-2	i.p.	generalization	0.28 (0.10–0.84)
Δ^9-THC	i.p.	generalization	0.34 (0.11–1.06)
	p.o.	generalization	0.43 (0.20–0.93)
SR + BAY 38-7271[b]	i.p.	antagonism	1.05 (0.56–1.98)

Abbreviations used: CL = confidence limits, NC = not computable, Δ^9-THC=(−)-Δ^9-tetrahydrocannabinol, SR = SR 141716A.
[a] Doses in mg/kg.
[b] 0.05 mg/kg, i.p.

As shown in Fig. 1, dose-dependent and complete generalization was also obtained after i.p. administration of the reference cannabinoid CB_1 receptor agonists HU-210, CP 55,940, WIN 55,212-2 and (−)-Δ^9-tetrahydrocannabinol (ED_{50} values in Table 1). CP 55,940 and (−)-Δ^9-tetrahydrocannabinol also generalized dose-dependently when tested after p.o. administration (data not shown, complete generalization at 0.1 mg/kg CP 55,940 and 3 mg/kg (−)-Δ^9-tetrahydrocannabinol, respectively, ED_{50} values in Table 1). For each of these compounds complete generalization could be obtained in the absence of behavioral disruption (100% Lever Selections). At the highest dose tested, HU-210 (0.03 mg/kg) and WIN 55,212-2 (3 mg/kg) induced signs of behavioral toxicity, as indicated by a 50% and 100% reduction in Lever Selections, respectively.

Generalization test results obtained with the five cannabinoid CB_1 receptor agonists in the BAY 38-7271 drug discrimination assay and the CP 55,940 drug discrimination assay (Mauler et al., 2002) were virtually identical ($r^2 = 0.98$, Fig. 4), whereas generalization data obtained with these compounds in the BAY 38-7271 drug discrimination assay correlated highly with binding affinity to cannabinoid CB_1

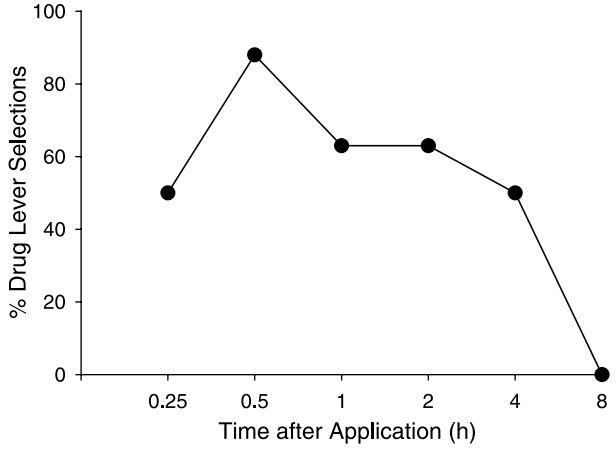

Fig. 2. Time-dependency of the discriminative stimulus induced by the training dose of BAY 38-7271 in rats trained to discriminate this compound from vehicle (0.05 mg/kg, i.p., $t-30$ min). Each time point was independently measured. $n = 8$ for each time point, except 30 min, i.p. ($n = 16$).

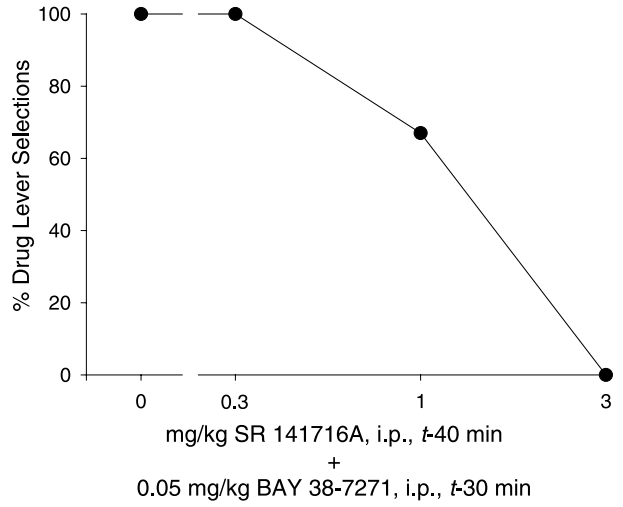

Fig. 3. Dose-dependent antagonism of the BAY 38-7271 cue by pretreatment with the selective cannabinoid CB_1 receptor antagonist SR 141716A (SR). SR 141716A was administered i.p., 10 min before BAY 38-7271 or vehicle. $n = 6$ per dose.

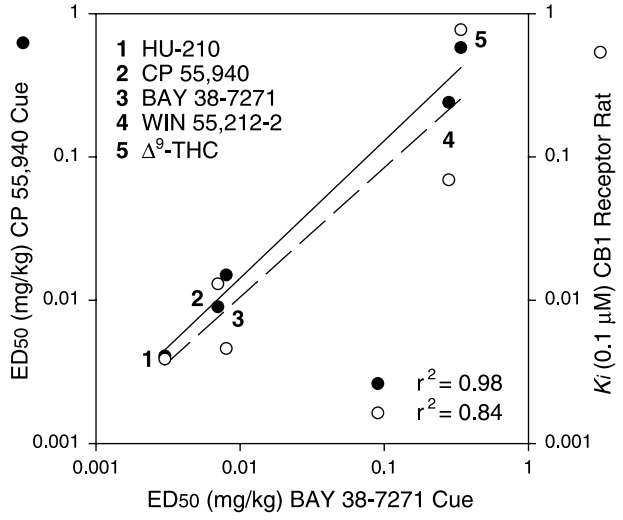

Fig. 4. Correlation between generalization test results (ED_{50} values after i.p. administration) obtained with cannabinoid CB_1 receptor agonists in the BAY 38-7271 drug discrimination assay and in a CP 55,940 drug discrimination assay (closed circles), and between generalization to the BAY 38-7271 cue and binding (K_i values) to cannabinoid CB_1 receptors (open circles). In the CP 55,940 drug discrimination assay, rats were trained to discriminate CP 55,940 (0.03 mg/kg, i.p., $t-30$ min) from vehicle, and subsequently tested for stimulus generalization under identical conditions as used for the BAY 38-7271 discrimination. Binding data were obtained using rat brain membranes and [^{3}H]BAY 38-7271 as radioligand. Data obtained in the CP 55,940 drug discrimination assay and the binding assay were adapted from Mauler et al. (2002, methods described in this reference). Δ^9-THC=(−)-Δ^9-tetrahydrocannabinol.

receptors labeled by [^{3}H]BAY 38-7271 ($r^2 = 0.84$, Fig. 4, binding assay methodology and results described in Mauler et al., 2002).

4. Discussion

The novel diarylether sulfonylester BAY 38-7271 was recently characterized as a high-affinity cannabinoid CB_1 receptor agonist ($K_i = 0.46–1.85$ nM) with neuroprotective properties (Mauler et al., 2002). In a former study (Mauler et al., 2002), the effects of BAY 38-7271 and the reference cannabinoid CB_1 receptor agonists CP 55,940, HU-210, WIN 55,212-2 and (−)-Δ^9-tetrahydrocannabinol were compared in the rat hypothermia assay and the rat CP 55,940 drug discrimination assay, two behavioral models sensitive to cannabinoid CB_1 receptor activation (Martin et al., 1991; Pertwee, 1984; Wiley et al., 1995b). Similar to the reference compounds, BAY 38-7271 was found to induce dose-dependent and SR 141716A-reversible effects in both models. These findings supported the notion that BAY 38-7271 is a highly potent cannabinoid CB_1 receptor agonist in vivo. The present characterization of the discriminative stimulus effects of BAY 38-7271 by means of generalization and antagonism tests further confirmed that the compound is a highly potent cannabinoid CB_1 receptor agonist in vivo.

In the present study, all rats successfully learned to discriminate a relatively low dose of BAY 38-7271 from vehicle. The training dose of BAY 38-7271 (i.e., 0.05 mg/kg) was selected to be equivalent in terms of stimulus "intensity" or "discriminability" to the training dose of CP 55,940 formerly used in a similar drug discrimination procedure (i.e., 0.03 mg/kg; Mauler et al., 2002). Thus, in the latter study it was found that the ED_{50} of stimulus generalization to the CP 55,940 cue was 0.009 and 0.015 mg/kg for CP 55,940 and BAY 38-7271, respectively (both tested 30 min after i.p. administration). Further evidence that the training dose of both compounds was indeed equivalent in intensity or discriminability is indicated by the fact that the median number of sessions to reach criterion was similar for both assays [i.e., 38 (range: 29–103) and 52 (range: 26–78) sessions for the CP 55,940 and BAY 38-7271 cue, respectively], and the finding that the ED_{50} of stimulus generalization of both compounds to the BAY 38-7271 cue was virtually identical to the values obtained in the CP 55,940 cue (i.e., 0.007 and 0.018 mg/kg for CP 55,940 and BAY 38-7271, respectively, both tested again 30 min after i.p. administration). As in the case of the CP 55,940 cue, complete generalization with the BAY 38-7271 cue was obtained with both compounds at doses which did not induce any sign of behavioral toxicity (i.e., 100% Lever Selections).

Generalization tests performed with various cannabinoid CB_1 receptor agonists indicated that each of these compounds generalized dose-dependently and completely to the BAY 38-7271 cue. Interestingly, the order of potency obtained with these compounds (i.e., HU-210 < CP 55,940 ≈ BAY 38-7271 < WIN 55212-2 ≈ (−)-Δ^9-tetrahydrocannabinol) was identical with that obtained in the CP 55,940 cue (Mauler et al., 2002; Wiley et al., 1995b). As shown in Fig. 4, not only the relative potency, but also the absolute potency of these compounds to generalize to the BAY 38-7271 cue and to generalize to the CP 55,940 cue (Mauler et al., 2002) is highly similar ($r^2 = 0.98$). This strongly suggests that the discriminative stimulus effects of both compounds are very similar, if not identical. It has been previously demonstrated that the discriminative stimulus induced by a cannabinoid CB_1 receptor agonist (i.e., (−)-Δ^9-tetrahydrocannabinol, WIN 55212-2 or CP 55,940) is highly sensitive and specific (Balster and Prescott, 1992; Barrett et al., 1995; Martin et al., 1991; Wiley, 1999; Wiley et al., 1995b). Thus, it was reported that rats, gerbils, pigeons or primates trained to discriminate a cannabinoid CB_1 receptor agonist from vehicle only showed complete generalization if tested with cannabinoid CB_1 receptor agonists, whereas compounds from other pharmacological classes failed to induce complete generalization (for review, see Balster and Prescott, 1992; Barrett et al., 1995; Wiley, 1999). The present finding that the ED_{50} values obtained with various cannabinoid CB_1 receptor agonists in the BAY 387271 assay correlated highly ($r^2 = 0.84$) with their K_i values obtained in a rat brain membrane binding assay using [^{3}H]BAY 38-7271 as radioligand (Mauler et al., 2002), again, strongly suggests that the BAY 38-7271 cue is mediated by activation of cannabinoid CB_1 receptors. This suggestion is also supported by the finding that the discriminative effects of BAY 38-7271 and CP 55,940 (as assessed in the BAY 38-7271 cue or in the CP 55,940 cue; Mauler et al., 2002) both could be blocked in a dose-dependent, complete and equipotent manner by the cannabinoid CB_1 receptor antagonist SR 141716A. The fact that the ID_{50} values obtained with SR 141716A to block the generalization to BAY 38-7271 (or CP 55,940) in both assays are virtually identical (i.e., 1.05 and 1.14 mg/kg, in the BAY 38-7271 cue and the CP 55,940 cue, respectively; Mauler et al., 2002) supports the suggestion that the "intensity", as well as, the quality of the discriminative stimulus effects of both compounds is highly similar. In general, the findings with SR 141716A are in accordance with other studies which reported that the discriminative effects of (−)-Δ^9-tetrahydrocannabinol, CP 55,940 or WIN 55212-2 could be blocked by the cannabinoid CB_1 receptor antagonist (De Vry and Jentzsch, 1999; Järbe et al., 2001; Mansbach et al., 1996; Pério et al., 1996; Wiley et al., 1995a,b).

As compared with the hypothermia assay (Mauler et al., 2002), the drug discrimination assay appears to be about 10–30 times more sensitive (comparison of potency differences after i.p. administration). This suggests that the cannabinoid CB_1 receptor population mediating the discriminative effects is different from, and possibly more efficiently coupled to effector systems than the cannabinoid CB_1 receptor population mediating the hypothermic effects (De Vry, 2002). With respect to the latter possibility, it

should be mentioned that it has already been demonstrated that a relatively large receptor reserve exists for some behavioral effects induced by cannabinoid CB_1 receptor agonists (Gifford et al., 1999).

Both in the present drug discrimination assay and the previously reported CP 55,940 drug discrimination assay (Mauler et al., 2002), it was found that BAY 38-7271 potently generalized after i.v. administration (ED_{50} values: 1 and 0.4 μg/kg, respectively). This finding suggests that activation of cannabinoid CB_1 receptors can be expected to occur at doses as low as 0.1–1 μg/kg, i.v. of the compound. Because the neuroprotective effects of BAY 38-7271 have been obtained in a similar dose range (Mauler et al., 2002), these data are in accordance with the hypothesis that cannabinoid CB_1 receptor agonist properties of the compound are responsible for the neuroprotective effects of the compound.

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