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Discriminative stimulus effects of the structurally novel cannabinoid CB_1/CB_2 receptor partial agonist BAY 59-3074 in the rat

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Abstract

BAY 59-3074 {3-[2-cyano-3-(trifluoromethyl)phenoxy]phenyl-4,4,4-trifluoro-1-butane-sulfonate} is a structurally novel cannabinoid CB_1/CB_2 receptor partial agonist with analgesic properties. The present study was performed to confirm its receptor binding profile in a highly sensitive in vivo assay. Rats (n=10) learned to discriminate BAY 59-3074 (0.5 mg/kg, p.o., $t-1$ h) from vehicle in a fixed-ratio: 10, food-reinforced two-lever procedure after a median number of 28 training sessions. BAY 59-3074 generalized dose-dependently (ED_{50}: 0.081 mg/kg, p.o.) and the cue was detectable between 0.25 and 4 h after administration. 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] blocked the discriminative effects of BAY 59-3074 (ID_{50}: 1.79 mg/kg, i.p.). Complete generalization was also obtained after i.p. administration of BAY 59-3074 (ED_{50} value: 0.41 mg/kg), and the reference cannabinoids BAY 38-7271 [(−)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-butanesulfonate, 0.011 mg/kg], CP 55,940 {(−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxy-propyl)cyclohexanol, 0.013 mg/kg}, HU-210 [(−)-11-OH-Δ^8-tetrahydrocannabinol dimethylheptyl, 0.022 mg/kg], 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, 0.41 mg/kg] and (−)-Δ^9-tetrahydrocannabinol (0.41 mg/kg). Non-cannabinoids with analgesic properties, such as morphine, amitriptyline, carbamazepine, gabapentin and baclofen, did not generalize to the cue. It is concluded that the discriminative stimulus effects of BAY 59-3074 are specifically mediated by cannabinoid CB_1 receptor activation.

Keywords: Analgesic; Animal model; Cannabinoid receptor; Drug discrimination

1. Introduction

BAY 59-3074 {3-[2-cyano-3-(trifluoromethyl)phenoxy]-phenyl-4,4,4-trifluoro-1-butanesulfonate} is a structurally novel cannabinoid with pronounced antihyperalgesic and anti-allodynic properties in rat models of chronic neuropathic and inflammatory pain (Fig. 1; De Vry et al., 2004c). In vitro, the compound was characterized as a cannabinoid CB_1 receptor ligand (K_i=48–55 nM, as assessed at human and rat cannabinoid CB_1 receptors), with partial agonist properties at this receptor as indicated by [^{35}S]GTPγS binding assays (De Vry et al., 2004c). Besides additional affinity for cannabinoid CB_2 receptors (K_i=45 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 one order of magnitude, suggesting that BAY 59-3074 is a relatively selective cannabinoid receptor agonist (De Vry et al., 2004c).

Behavioral studies have suggested that the compound is also an orally active cannabinoid CB_1 receptor agonist in vivo (De Vry et al., 2004c). Thus, in rats trained to discriminate the highly potent, selective and structurally related cannabinoid CB_1 receptor full agonist BAY 38-7271 [(−)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-butanesulfonate] (Fig. 1; Mauler et al., 2002) from vehicle, BAY 59-3074 induced complete generalization

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(ED_{50}: 0.24 and 0.17 mg/kg, after i.p. and p.o. administration, respectively). As it has been proposed that the discriminative stimulus effects of BAY 38-7271 are mediated by activation of cannabinoid CB_1 receptors (De Vry and Jentzsch, 2002; Mauler et al., 2002), this result supports the notion that BAY 59-3074 is a cannabinoid CB_1 receptor agonist in vivo. In addition, BAY 59-3074 was found to induce a reduction in body temperature at higher doses (≥5 mg/kg, p.o., De Vry et al., 2004c). As such hypothermic effects have also been obtained with other cannabinoid CB_1 receptor agonists (De Vry et al., 2004a; Martin et al., 1991; Mauler et al., 2002; Pertwee, 1984), this finding is compatible with its characterization as a cannabinoid CB_1 receptor agonist. Moreover, it was demonstrated in both in vivo models that the effects of BAY 59-3074 and other cannabinoids were 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 59-3074, and 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 (0.5 mg/kg, p.o.) from vehicle in a standard two-lever food-reinforced drug discrimination procedure, and subsequently the effects of BAY 59-3074 were compared with those of the reference cannabinoid CB_1 receptor agonists CP 55,940 {(−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol, Johnson and Melvin, 1986}, HU-210 [(−)-11-OH-Δ^8-tetrahydrocannabinol dimethylheptyl, Järbe et al., 1989], 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, Compton et al., 1992], (−)-Δ^9-tetrahydrocannabinol (Gaoni and Mechoulam, 1964), and BAY 38-7271 in generalization test sessions. In order to further confirm the involvement of cannabinoid CB_1 receptors, it was tested whether pretreatment with SR 141716A was able to block the discriminative effects of BAY 59-3074. Finally, it was investigated whether the discriminative effects of BAY 59-3074 could be differentiated from those of compounds generally used for the treatment of chronic pain, such as the opiate morphine, the tricyclic antidepressant amitriptyline, the anticonvulsants carbamazepine and gabapentin, and the muscle relaxant baclofen (Sindrup and Jensen, 1999).

2. Material and methods

2.1. Animals

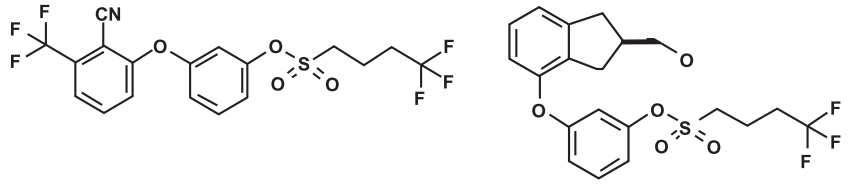
Male Wistar rats (n=10) were purchased from Harlan-Winkelmann (HsdCpb: WU, Borchen, Germany). Body weight upon arrival at the laboratory was around 160 g, which gradually increased up to about 500 g during the course of the study. Rats were individually housed in Makrolon® type 3 cages under a normal 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, 2002) was followed. After initial shaping to lever press for food reinforcement, the rats were trained to discriminate BAY 59-3074 (0.5 mg/kg, p.o., $t-1$ h) from vehicle in a standard two-lever, fixed ratio: 10 operant procedure. Daily sessions were conducted which were



BAY 59-3074 BAY 38-7271

Fig. 1. Structures of BAY 59-3074 and BAY 38-7271.

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 59-3074, 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. All animals were tested first with different doses of BAY 59-3074 in a random sequence (0 and 0.031–0.5 mg/kg, p.o.; $t-1$ h; 0 and 0.3–1 mg/kg, i.p.; $t-0.5$ h). In the time-dependency study, BAY 59-3074 was tested 0.25, 0.5, 1, 2 and 4 h following p.o. administration of 0.5 mg/kg, during different test sessions. Further generalization tests were performed with the reference cannabinoid CB_1 receptor agonists CP 55,940 (0.003–0.03 mg/kg), HU-210 (0.01–0.03), WIN 55,212-2 (0.3–1 mg/kg), Δ^9-tetrahydrocannabinol (0.3–1 mg/kg) and BAY 38-7271 (0.006–0.05 mg/kg), and with the non-cannabinoids morphine (3–10 mg/kg), amitriptyline (3–30 mg/kg), carbamazepine (30–60 mg/kg), gabapentin (30–100 mg/kg) and baclofen (1–3 mg/kg). All these compounds were tested i.p., 0.5 h after administration (except for HU-210 and gabapentin, which were tested after 2 and 1 h, respectively). Δ^9-tetrahydrocannabinol was also tested after p.o. administration (0.3–3 mg/kg, $t-1$ h) and in a time-dependency study (3 mg/kg, p.o., tested 0.25, 0.5, 1 and 2 h after administration, during different sessions). In the antagonism studies, pretreatment with SR 141716A (1–5 mg/kg, or vehicle; i.p.) occurred 1 h before treatment with BAY 59-3074 (0.5 mg/kg or vehicle, p.o.; $t-1$ h). In general, each dose of each test compound or test compound combination was tested in five rats, randomly selected for each test condition from the 10 rats which successfully reached the discrimination criterion.

2.4. Drugs

BAY 59-3074 {3-[2-cyano-3-(trifluoromethyl)phenoxy]-phenyl-4,4,4-trifluoro-1-butane-sulfonate}, BAY 38-7271 [(−)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-butanesulfonate], 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 HealthCare (Wuppertal, Germany). Other compounds included (−)-Δ^9-tetrahydrocannabinol, carbamazepine and baclofen (Sigma-Aldrich, Steinheim, Germany), WIN 55,212-2 [(*R*)-4,5-dihydro-2-methyl-4(4-morpholinyl-methyl)-1-(1-naphthalenylcarbonyl)-6*H*-pyrrolo[3,2,1-*ij*]quinolin-6-one] and amitriptyline (RBI, Natick, MA, USA), morphine hydrochloride (Merck, Darmstadt, Germany), and gabapentin (content of capsules of Neurontin®, Parke-Davis, Freiburg, Germany, extracted by the Medical Chemistry Department, Bayer HealthCare). BAY 59-3074 and gabapentin were suspended in distilled water and 5% or 10% Cremophor® EL (BASF, Ludwigshafen, Germany), respectively. Carbamazepine was suspended in a solvent containing 2% DMSO (dimethylsulfoxide; Merck), 10% cremophor and distilled water. BAY 38-7271, BAY 38-7271, HU-210, CP 55,940, WIN 55,212-2, Δ^9-tetrahydrocannabi-

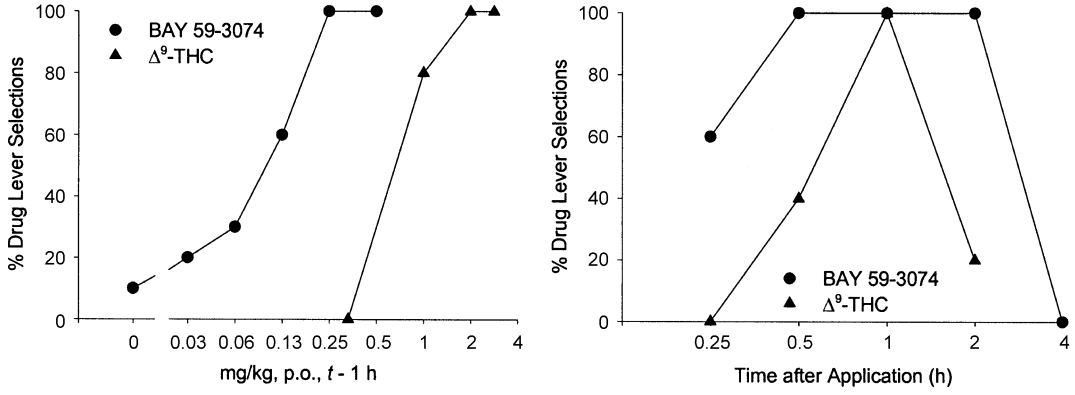


Fig. 2. Dose-dependency (left panel) and time-dependency (right panel) of generalization induced by oral administration of BAY 59-3074 and Δ^9-tetrahydrocannabinol (Δ^9-THC) in rats trained to discriminate BAY 59-3074 (0.5 mg/kg, p.o, $t-1$ h) from vehicle. The time-dependency study was performed with 0.5 mg/kg BAY 59-3074 and 3 mg/kg Δ^9-THC, and each time point was independently measured. Dose-dependency study: n=10 and 5, per dose, for BAY 59-3074 and Δ^9-THC, respectively. Time-dependency study: n=5 per time point, except 1 h, BAY 59-3074 (n=10).

Table 1
Summary of test results with cannabinoid CB_1 receptor ligands in rats trained to discriminate BAY 59-3074 from vehicle

Compound (K_i in nM, cannabinoid CB_1 receptor)[a]	Application	Type of test	% Drug lever selections ED_{50} (95% CL)[b]
BAY 59-3074	p.o.	generalization	0.081 (0.047–0.142)
(55.4 nM)	i.p.	generalization	0.41 (0.26–0.66)
HU-210 (0.4 nM)	i.p.	generalization	0.022 (0.015–0.034)
CP 55,940 (1.2 nM)	i.p.	generalization	0.013 (0.009–0.021)
BAY 38-7271 (0.5 nM)	i.p.	generalization	0.011 (0.006–0.020)
WIN 55,212-2 (6.9 nM)	i.p.	generalization	0.41 (0.26–0.66)
Δ^9-THC	i.p.	generalization	0.41 (0.26–0.66)
(73.5 nM)	p.o.	generalization	0.53 (0.25-1.11)
SR+BAY 59-3074[c]	i.p.	antagonism	1.79 (0.91–3.53)

CL=confidence limits, Δ^9-THC=Δ^9-tetrahydrocannabinol, SR=SR 141716A.
[a] K_i values obtained at rat brain membranes with [^{3}H]BAY 38-7271 as radioligand (adapted from Mauler et al., 2002 and De Vry et al., 2004c).
[b] Doses in mg/kg.
[c] 0.5 mg/kg, p.o.

nol and SR 141716A were suspended in a solvent containing 1–2.5% Solutol® HS 15 (12-hydroxystearic-acid ethoxylate, BASF), 1–2.5% ethanol (ethanol absolute, 99.8%, Riedel-de Haën, Seelze, Germany) and distilled water or 0.9% NaCl (saline). Morphine and amitriptyline were dissolved in saline and distilled water, respectively. 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). 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}, ID_{50} and $T_{1/2}$ values (and their 95% confidence limits) after log-probit conversion of the data. Generalization and antagonism was considered to be complete if at least 80% and less than 20% drug lever selections was obtained, respectively. In order to compare the generalization data obtained with the various cannabinoids in the present drug discrimination with those obtained previously in a BAY 38-7271 drug discrimination (both assays using the same experimental conditions, De Vry and Jentzsch, 2002; De Vry et al., 2004c), a linear regression analysis was performed on the respective $\log_{10}$-transformed ED_{50} values.

3. Results

All 10 rats learned to discriminate BAY 59-3074 (0.5 mg/kg, p.o.) from vehicle, the median number of sessions to reach criterion being 28 (range: 25–43 sessions). The generalization obtained with BAY 59-3074 was dose-dependent, with complete generalization at 0.25–0.5 mg/kg (Fig. 2, left panel, ED_{50} value in Table 1), and occurred in the absence of behavioral disruption (indicated by the occurrence of 100% Lever Selections at each dose).

A time-dependency study indicated that the discriminative effects of BAY 59-3074 (0.5 mg/kg) reached maximal intensity between 0.5 and 2 h after p.o. administration, and disappeared within 4 h ($T_{1/2}$: 170 min, 100% Lever Selections at each dose, Fig. 2, right panel). Pretreatment with SR 141716A dose-dependently attenuated the discriminative effects of BAY 59-3074, with complete antagonism at 5 mg/kg (Fig. 3, left panel, ID_{50} value in Table 1). SR 141716A (1–5 mg/kg) did not induce generalization when tested alone (0% Drug Lever elections at each dose). Again, all rats selected a lever, under each test condition.

As shown in Fig. 3 (right panel), dose-dependent and complete generalization was obtained after i.p. administration of BAY 59-3074 and the reference cannabinoids CP

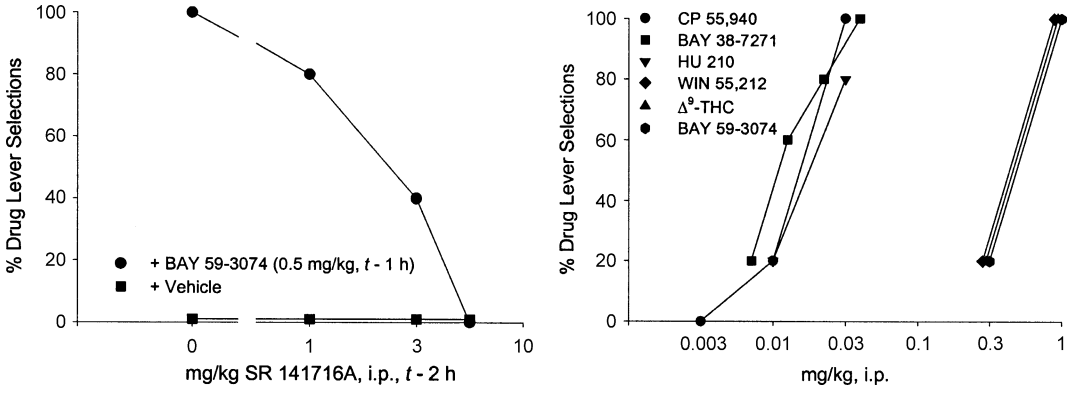


Fig. 3. Left panel: dose-dependent antagonism of the discriminative stimulus induced by BAY 59-3074 (0.5 mg/kg, p.o., $t-1$ h) by pretreatment with the selective cannabinoid CB_1 receptor antagonist SR 141716A (i.p., administered 1 h before BAY 59-3074). Right panel: Dose-dependent generalization induced by structurally diverse cannabinoid CB_1 receptor agonists. Compounds were administered i.p., 0.5 h before test, except for HU-210, which was administered 2 h before test. Vehicle induced 0% Drug Lever Selections (i.p., $t-0.5$ h). n=5 per dose.

Table 2
Generalization test results obtained with non-cannabinoid compounds with analgesic properties

Compound	Dose (mg/kg)	% Drug lever selections	% Lever selections
Morphine	3	0%	100%
	5.5	0%	100%
	10	NC	0%
Amitriptyline	3	0%	100%
	10	40%	100%
	30	0%	60%
Carbamazepine	30	20%	100%
	60	25%	80%
Gabapentin	30	20%	100%
	100	0%	100%
Baclofen	1	0%	100%
	3	33%	60%

Compounds were administered i.p., 0.5 h before test, except for gabapentin, which was administered 1 h before test. Vehicle induced 0% Drug Lever Selections and 100% Lever Selections. n=4–5 per dose.
NC=not computable.

55,940, BAY 38-7271, HU-210, WIN 55,212-2 and Δ^9-tetrahydrocannabinol (ED_{50} values in Table 1). For each of these compounds generalization occurred in the absence of behavioral disruption (indicated by the occurrence of 100% Lever Selections at each dose). Generalization test results (represented by the ED_{50} values) obtained after i.p. administration of the six cannabinoids in the BAY 59-3074 and the BAY 38-7271 drug discrimination assay (De Vry and Jentzsch, 2002; De Vry et al., 2004c) were virtually identical (r^2=0.85, P<0.01). Δ^9-Tetrahydrocannabinol also generalized dose-dependently when tested after p.o. administration, with complete generalization at 2–3 mg/kg (100% Lever Selections at each dose, Fig. 2, left panel, ED_{50} value in Table 1). The time-dependency study with Δ^9-tetrahydrocannabinol (3 mg/kg) indicated that the discriminative effects of the compound were maximal 1 h after p.o. administration, and disappeared within 2 h (100% Lever Selections at each time point, $T_{1/2}$: 85 min, Fig. 2, right panel).

Tests with morphine, amitriptyline, carbamazepine, gabapentin and baclofen indicated that none of these compounds generalized to the BAY 59-3074 cue (Table 2). In general, these compounds were tested up to doses that showed behavioral disruption (i.e., decrease in % Lever Selections).

4. Discussion

BAY 59-3074 was recently characterized as a structurally novel cannabinoid CB_1/CB_2 receptor partial agonist with pronounced antihyperalgesic and anti-allodynic properties in rat models of chronic neuropathic and inflammatory pain (De Vry et al., 2004c). In that study, the effects of BAY 59-3074 and the reference cannabinoid CB_1 receptor agonists CP 55,940 and Δ^9-tetrahydrocannabinol were compared after oral administration in the rat hypothermia assay and the rat BAY 38-7271 drug discrimination assay, two behavioral models sensitive to cannabinoid CB_1 receptor activation (De Vry and Jentzsch, 2002; Martin et al., 1991; Mauler et al., 2002; Pertwee, 1984). Similar to the reference compounds, BAY 59-3074 was reported to induce dose-dependent and SR 141716A-reversible effects in both models. The present characterization of the discriminative stimulus effects of BAY 59-3074 by means of generalization and antagonism tests further confirmed that the compound is an orally active cannabinoid CB_1 receptor agonist.

In the present study, all rats successfully learned to discriminate a relatively low dose of BAY 59-3074 from vehicle. Selection of the training dose of BAY 59-3074 (i.e., 0.5 mg/kg p.o.) was intended to be equivalent in terms of stimulus "intensity" or "dicriminability" to the training dose of BAY 38-727 formerly used in a similar drug discrimination procedure (i.e. 0.05 mg/kg, i.p.; De Vry et al., 2004c). It was found that the ED_{50} of stimulus generalization of BAY 59-3074 (tested 1 h after p.o. administration) to either BAY 59-3074 cue or the BAY 38-7271 cue was 0.81 and 0.17 mg/kg, respectively; whereas ED_{50} of stimulus generalization of BAY 38-7271 (tested 0.5 h after i.p. administration) to either cue was 0.011 and 0.018 mg/kg, respectively. As it is generally accepted that the stimulus intensity positively correlates with the dose of the drug stimulus, it is possible that the stimulus intensity of the BAY 59-3074 cue was slightly higher than that of the BAY 38-7271 cue. This suggestion is further supported by the fact that the median number of sessions to reach criterion tended to be lower in the case of the BAY 59-3074 cue [i.e., 28 (range: 25–43) and 52 (range: 26–78) sessions for the BAY 59-3074 and BAY 38-7271 cue, respectively]. As in the case of the BAY 38-7271cue, complete generalization with the BAY 59-3074 cue was obtained with either compound at doses which did not induce any sign of behavioral disruption (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 completeley to the BAY 59-3074 cue. Interestingly, the order of potency obtained with these compounds after i.p. administration (i.e., BAY 38-7271 ≈ CP 55,940 ≤ HU-210 < BAY 59-3074 ≈ WIN 55212-2 ≈ Δ^9-tetrahydrocannabinol) was similar to that obtained in both the BAY 38-7271 cue and the CP 55,940 cue (De Vry and Jentzsch, 2002; Mauler et al., 2002; Wiley et al., 1995b). Moreover, not only the relative potency, but also the absolute potency of these compounds generalized to BAY 59-3074 cue and to generalize to the BAY 38-7271 cue (De Vry and Jentzsch, 2002; De Vry et al., 2004c) appeared to be highly similar (r^2=0.85). This strongly suggests that the discriminative stimulus effects of these structurally diverse 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, CP 55,940, or BAY 38-7271) is highly sensitive and specific (Balster and Prescott, 1992; Barrett et al., 1995; De Vry and Jentzsch, 2002; 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 59-3074 assay (i.p. administration) correlated highly (r^2=0.82, P<0.02) with their K_i values obtained in a rat brain membrane binding assay using [^{3}H]BAY 38-7271 as radioligand (Table 1; De Vry et al., 2004c; Mauler et al., 2002), again, strongly suggests that the BAY 59-3074 cue is mediated by activation of cannabinoid CB_1 receptors. This suggestion is also supported by the finding that the discriminative effects of BAY 59-3074 (as assessed in the BAY 59-3074 cue or in the BAY 38-7271 cue, De Vry et al., 2004c) could be completely blocked by the selective cannabinoid CB_1 receptor antagonist SR 141716A. 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, WIN 55212-2 or BAY 38-7271 are blocked by this compound (De Vry and Jentzsch, 2002, 2003; Järbe et al., 2001; Mansbach et al., 1996; Mauler et al., 2002; Pério et al., 1996; Wiley et al., 1995a,b). The fact that a variety of chemically diverse cannabinoids generalize to each other, in an SR141716A-reversible manner and with a similar order of potency, strongly indicates that the discriminative effects of these compounds are mediated by a common mechanism of action, which most likely involves activation of the cannabinoid CB_1 receptor subtype.

Further evidence that the BAY 59-3074 cue is pharmacologically specific is indicated by the finding that the pharmacologically diverse reference compounds clinically used for the treatment of chronic pain failed to generalize to the cue (for review of clinical efficacy, see Sindrup and Jensen, 1999). Thus, neither the opiate morphine, the tricyclic antidepressant amitriptyline, the anticonvulsants carbamazepine and gabapentin, nor the muscle relaxant baclofen induced more than, at best, partial generalization (maximal level of generalizion: 40% Drug Lever Selections, induced by amitriptyline). In general, these compounds were tested up to doses that showed behavioral disruption, and generalization tests covered a dose range which shows efficacy in rat models of chronic neuropathic pain (e.g., De Vry et al., 2004b; Hofmann et al., 2003).

As it was found that the BAY 59-3074 cue (1) generalized completely to various cannabinoid CB_1 receptor agonists with a potency order that closely resembled that obtained in other cannabinoid CB_1 receptor-sensitive in vitro assays, such as the cannabinoid CB_1 receptor binding or [^{35}S]GTPγS binding assay, and in vivo assays, such as the hypothermia assay or the BAY 38-7271 drug discrimination assay (De Vry et al., 2004c; Mauler et al., 2002), (2) could be completely blocked by the cannabinoid CB_1 receptor antagonist SR 141716A, and (3) failed to generalize to analgesics from various (non-cannabinoid) pharmacological classes, it is concluded that the discriminative stimulus effects of BAY 59-3074 are specifically mediated by cannabinoid CB_1 receptor activation.

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