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O-1057, a potent water-soluble cannabinoid receptor agonist with antinociceptive properties

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1 Cannabinoids have low water solubility, necessitating the use of a solubilizing agent. In this paper we investigated whether a novel water-soluble cannabinoid, 3-(5′-cyano-1′,1′-dimethylpentyl)-1-(4-N-morpholinobutyryloxy)-Δ^8-tetrahydrocannabinol hydrochloride (O-1057), would interact with cannabinoid receptors when water or saline were used as the only vehicle.

2 O-1057 displaced [^{3}H]-CP55940 from specific binding sites on Chinese hamster ovary (CHO) cell membranes expressing CB_1 or CB_2 cannabinoid receptors, with pK_i values of 8.36 and 7.95 respectively. It also displaced [^{3}H]-CP55940 from specific binding sites on rat brain membranes ($pK_i = 7.86$).

3 O-1057 inhibited forskolin-stimulated cyclic AMP production by both CB_1- and CB_2-transfected CHO cells ($pEC_{50} = 9.16$ and 9.72 respectively), its potency matching that of CP55940 and exceeding that of Δ^9-tetrahydrocannabinol.

4 In the mouse isolated vas deferens, O-1057 inhibited electrically-evoked contractions with pEC_{50} and E_{max} values of 9.73 and 76.84% respectively. It was antagonized by 100 nM SR141716A, the pK_B of SR141716A against O-1057 (8.90) approximating to that against CP55940 (8.97).

5 O-1057 also behaved as a CB_1 receptor agonist *in vivo*, reducing mouse spontaneous activity and rectal temperature when injected intravenously and inducing antinociception in the mouse tail flick test when given intravenously ($ED_{50} = 0.02$ mg kg^{-1}), intrathecally, intracerebroventricularly or by gavage. In all these assays, O-1057 was more potent than Δ^9-tetrahydrocannabinol and, at 0.1 mg kg^{-1} i.v., was antagonized by SR141716A (3 mg kg^{-1} i.v.).

6 These data demonstrate the ability of the water-soluble cannabinoid, O-1057, to act as a potent agonist at CB_1 and CB_2 receptors and warrant investigation of the clinical potential of O-1057 as an analgesic.

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Abbreviations: BSA, bovine serum albumin; CHO, Chinese hamster ovary; CP55940, (−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol; IBMX, 3-isobutyl-1-methylxanthine; MPE, maximum possible effect; O-1057, 3-(5′-cyano-1′,1′-dimethylpentyl)-1-(4-N-morpholinobutyryloxy)-Δ^8-tetrahydrocannabinol hydrochloride; SR141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride; Δ^9-THC, Δ^9-tetrahydrocannabinol

Introduction

The cloning of cannabinoid CB_1 and CB_2 receptors in the early 1990's (Matsuda *et al*., 1990; Munro *et al*., 1993) has led to the development of an ever growing range of selective CB_1 and CB_2 receptor agonists and antagonists, for use as experimental tools (Pertwee, 1999). It has also sparked a renewed interest in the therapeutic potential of cannabinoids, for example for the relief of chronic pain (Joy *et al*., 1999). One major practical difficulty encountered in experiments with the cannabinoid receptor ligands that are currently available is their extremely low water solubility as this necessitates the use of a solubilizing agent such as polyethylene glycol, ethanol, dimethylsulphoxide or Tween 80 that itself tends to be pharmacologically active. This difficulty is also hindering the full exploitation of cannabinoids as therapeutic agents, particularly when delivery of the drug is to be by injection or by aerosol inhalation. There is, therefore, a need for water-soluble cannabinoid receptor ligands. A description of such compounds is already in the literature (Zitko *et al*., 1972; Razdan *et al*., 1976). However, these water-soluble cannabinoids were developed before the discovery of cannabinoid receptors and were, therefore, characterized only on the basis of their behavioural pharmacology. In this paper we describe the first pharmacological characterization of another water-soluble cannabinoid, O-1057 [3-(5′-cyano-1′,1′-dimethylpentyl)-1-(4-N-morpholinobutyryloxy)-Δ^8-tetrahydrocannabinol hydrochloride (Figure 1)]. This is an analogue of 1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol, (Singer *et al*., 1998) and has been designed to be more potent than Δ^9-tetrahydrocannabinol. It has been rendered water-soluble by addition of a 4-N-morpholinobutyryloxy moiety to the phenolic hydroxyl group that is located at position 1 of the aromatic ring of the parent compound.

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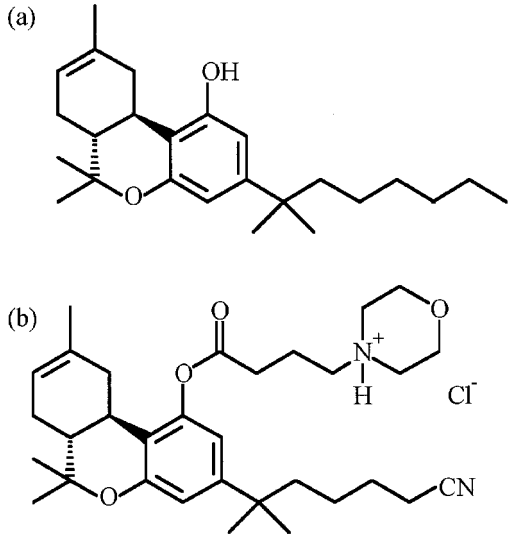

Figure 1 The structures of (a) 1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol and (b) O-1057.

Solubility has been further enhanced by the insertion of a cyano group in the dimethylpentyl side chain.

The pharmacology of O-1057 was explored by determining its affinity for CB_1 and CB_2 receptors, and by addressing the question of whether it shares the ability of cannabinoid receptor agonists (1) to inhibit forskolin-stimulated cyclic AMP production by CB_1 or CB_2 transfected cells, (2) to decrease the amplitude of electrically-evoked contractions of the mouse isolated vas deferens and (3) to reduce spontaneous activity, lower rectal temperature and induce antinociception in the tail flick test when administered intravenously to mice (Martin *et al*., 1995; Pertwee, 1999). The antinociceptive activity of O-1057 was also determined in the tail flick test following oral, intrathecal or intracerebroventricular administration. We compared O-1057 in these bioassays with CP55940 or Δ^9-tetrahydrocannabinol (Δ^9-THC), both established cannabinoid receptor agonists (Pertwee, 1999). We also determined the ability of the CB_1-selective competitive surmountable antagonist/inverse agonist, SR142716A (Rinaldi-Carmona *et al*., 1994; Pertwee, 1999), to oppose effects of O-1057 both on evoked contractions of the vas deferens and on spontaneous activity, rectal temperature and nociception.

Methods

CHO cells

These were stably transfected with cDNA encoding human CB_1 ($B_{max}=3.41$ pmol mg^{-1} protein) or CB_2 receptors ($B_{max}=31.4$ pmol mg^{-1} protein). They were provided by Drs G. Disney and A. Green, GlaxoWellcome R & D, Medicines Research Centre, Stevenage and were the same clones as those used in the sPAP reporter assay described by Green *et al*. (1999). Untransfected CHO cells were obtained from the Centre for Applied Microbiology and Research, Porton Down. Cells were maintained at 37°C and 5% CO_2 in DMEM/f-12 HAM with 2 mM glutamine, geneticin (600 μg ml^{-1}) and hygromycin (300 μg ml^{-1}). For binding experiments, CHO cells were removed from flasks by scraping and pellets frozen at −80°C for up to one month. Once defrosted the cell membranes were diluted to 1 mg ml^{-1} in 50 mM Tris buffer (pH 7.4) and resuspended using a hand-held homogenizer.

Binding experiments with CHO cell membranes

A modification of the filtration procedure described by Compton *et al*. (1993) was used. The assays were performed in the Aberdeen laboratory with [^{3}H]-CP55940, 1 mM $MgCl_2$, 1 mM EDTA, 1 mg ml^{-1} bovine serum albumin (BSA) and 50 mM Tris buffer, total assay volume 500 μl. Binding was initiated by the addition of CB_1 (100 μg protein) or CB_2 cell membranes (50 μg protein). Assays were carried out at 37°C for 30 min before termination by addition of ice-cold wash buffer (50 mM Tris buffer, 1 mg ml^{-1} BSA) and vacuum filtration using a 12-well sampling manifold (Brandel Cell Harvester) and Whatman GF/B glass-fibre filters that had been soaked in wash buffer at 4°C for 24 h. Each reaction tube was washed three times with a 4 ml aliquot of buffer. The filters were oven-dried for 60 min and then placed in 5 ml of scintillation fluid (Ultima Gold XR, Packard). Radioactivity was quantified by liquid scintillation spectrometry. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 1 μM unlabelled CP55940. Protein assays were performed using a Bio-Rad Dc kit. Unlabelled cannabinoids were each added in a volume of 50 μl after serial dilution from a 1 mg ml^{-1} stock solution using assay buffer (50 mM Tris buffer containing 5 mg ml^{-1} BSA). This stock solution was aqueous for O-1057 and ethanolic for CP55940. [^{3}H]-CP55940 was also added in a 50 μl volume following dilution in assay buffer. The concentration of [^{3}H]-CP55940 used in displacement assays was 0.5 nM. The concentrations of cannabinoids that produced a 50% displacement of radioligand from specific binding sites (IC_{50} values) were calculated using GraphPad Prism (GraphPad Software, San Diego, U.S.A.). Dissociation constant (K_i) values were calculated using the equation of Cheng and Prusoff (1973) and K_D values for [^{3}H]-CP55940 of 1.62 (CB_1) or 0.99 nM (CB_2). These K_D values were obtained in saturation binding assays using membranes from CB_1- or CB_2-transfected CHO cells as described previously (Ross *et al*., 1999).

Binding experiments with rat brain membranes

[^{3}H]-CP55940 ($K_D=690$ pM) binding to P_2 membranes was conducted in the laboratory of Dr Martin as described elsewhere (Compton *et al*., 1993), except that whole brain (rather than cerebral cortex only) was used. Displacement curves were generated by incubating drugs with 1 nM of [^{3}H]-CP-55,940. The assays were performed in triplicate, and the results represent the combined data from three individual experiments. The K_i values were determined from displacement data using EBDA (Equilibrium Binding Data Analysis; Biosoft, Milltown, NJ, U.S.A.). The K_D value of [^{3}H]-CP55940 obtained in saturation binding assays with rat brain membranes is somewhat less than the value obtained using membranes from CB_1-transfected CHO cells. It should be noted, therefore, that the two sets of experiments were performed in different laboratories and also that both values fall within the range of published K_D values for [^{3}H]-CP55940, 0.4–3.3 nM for the hCB_1 receptor and 0.07–2.3 nM for cannabinoid receptors on rat brain membranes (Pertwee, 1999).

Cyclic AMP assay

This was performed using the method described by Ross *et al*. (1999). Cells were preincubated for 20 min at 37°C with cannabinoid agonist and 3-isobutyl-1-methylxanthine (IBMX; 50 μM) in phosphate buffered saline containing 1 mg ml^{-1}

BSA (assay buffer) followed by a further 20 min incubation with 2 μM forskolin in a total volume of 500 μl. For antagonist studies, cells were preincubated for 10 min with the antagonist before agonist incubation. The reaction was terminated by addition of 0.1 M HCl and centrifugation performed to remove cell debris. The pH was brought to 8–9 using 1 M NaOH, and cyclic AMP content was then measured using a radioimmunoassay kit (Biotrak, Amersham, U.S.A.). O-1057 was dissolved in water and CP55940 in ethanol as 1 mg ml^{-1} stock solutions and these were diluted in assay buffer. Forskolin and IBMX were dissolved in DMSO. Cyclic AMP concentrations in CB_1-transfected cells were 21.80 ± 6.50 pmol ml^{-1} in the presence of 2 μM forskolin and 2.83 ± 1.01 pmol ml^{-1} in the absence of forskolin ($n = 11$). Corresponding values in CB_2-transfected cells were 18.24 ± 5.81 and 3.00 ± 1.27 pmol ml^{-1} respectively ($n = 11$).

Mouse vas deferens

Vasa deferentia were obtained from albino MF1 mice weighing 36–62 g. Each tissue was mounted in a 4 ml organ bath at an initial tension of 0.5 g. The baths contained Mg^{2+}-free Krebs solution which was kept at 37°C and bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs solution was (mM): NaCl 118.2, KCl 4.75, KH_2PO_4 1.19, $NaHCO_3$ 25.0, glucose 11.0 and $CaCl_2 \cdot 6H_2O$ 2.54. Isometric contractions were evoked by stimulation with 0.5 s trains of three pulses of 110% maximal voltage (train frequency 0.1 Hz; pulse duration 0.5 ms) through a platinum electrode attached to the upper end and a stainless steel electrode attached to the lower end of each bath. Stimuli were generated by a Grass S48 stimulator, then amplified (Med-Lab channel attenuator) and divided to yield separate outputs to four organ baths (Med-Lab StimuSplitter). Contractions were monitored by computer (Apple Macintosh LCIII and Performa 475) using a data recording and analysis system (MacLab) that was linked *via* preamplifiers (Macbridge) to Dynamometer UF1 transducers (Pioden Controls). Each tissue was subjected to several 5-min periods of stimulation, the first of these beginning after the tissue had equilibrated but before drug administration. The stimulator was switched off for 25 min between each 5-min stimulation period. Concentration-response curves for O-1057 and CP55940 were constructed either cumulatively, by adding one or other drug immediately after each 5-min stimulation period, or non-cumulatively by making only one addition of cannabinoid to each preparation. In some experiments, concentration-response curves for O-1057 or CP55940 were constructed in tissues that had been pretreated with SR141716A. In these experiments, SR141716A or vehicle was administered after the first 5 min stimulation period and O-1057 or CP55940 after the second stimulation period. For addition to organ baths O-1057 was dissolved in water whereas SR141716A and CP55940 were mixed with two parts of Tween 80 by weight and dispersed in a 0.9% aqueous solution of NaCl (saline) as described previously for Δ^9-tetrahydrocannabinol (Pertwee *et al*., 1992). Drug additions were made in a volume of 10 μl. In control experiments, Tween 80 was added instead of SR141716A or CP55940. The control dose of Tween 80 was the same as the dose added in combination with the highest cannabinoid dose used.

In vivo *pharmacology*

Male ICR mice (Harlan Laboratories, Dublin, VA, U.S.A.) weighing 18–25 g were maintained on a 14 : 10 h light–dark cycle with free access to food and water. For intravenous administration, Δ^9-THC was dissolved in 1 : 1 : 18 (emulphor–ethanol–saline), whereas O-1057 was dissolved in saline. The mice were injected in the tail vein at a volume of 0.01 ml g^{-1} of body weight. Following drug administration each animal was tested for effects on the following procedures: spontaneous (locomotor) activity at 5–15 min, tail-flick latency (antinociception) response at 20 min, and rectal temperature at 30 min. For intrathecal administration, Δ^9-THC was dissolved in 10% ethanol in DMSO and O-1057 was prepared in saline. A 5 μl solution was injected into the spinal column between L5 and L6 of unanaesthesized mice with a 30-gauge, one-half inch needle (Hylden & Wilcox 1980). The animals were tested for tail-flick response 5 min after the injection. Intracerebroventricular injections were performed as described previously (Welch *et al*., 1998). Mice were lightly anaesthetized with ether, and an incision was made in the scalp such that the bregma was exposed. Injections were performed using a 26-gauge needle with a sleeve of PE 20 tubing to control the depth of the injection. An injection (5 μl) was made 2 mm lateral and 2 mm caudal to the bregma at a depth of 2 mm. The vehicles for these injections were the same as those for intrathecal administration. The animals were tested 10 min after the injection.

Spontaneous activity was monitored by placing mice into individual activity cages (17×28 cm), and recording interruptions of photocell beams (16 beams per chamber) for a 10-min period using a Digiscan Animal Activity Monitor (Omnitech Electronics Inc., Columbus, Ohio, U.S.A.). Activity in the chamber was expressed as the total number of beam interruptions. Antinociception was assessed using the radiant heat tail-flick procedure. The heat lamp of the tail-flick apparatus was maintained at an intensity sufficient to produce control latencies of 2–3 s. Control values for each animal were determined prior to drug administration. Mice were then re-evaluated following drug administration and latency (time) to tail-flick response was recorded. A 10 s maximum was imposed to prevent tissue damage. The degree of antinociception was expressed as the per cent MPE (maximum possible effect) which was calculated as per cent MPE = [(test latency − control latency)/(10 − control)] × 100. Hypothermia was assessed by first measuring baseline core temperatures prior to drug treatment with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.) and a rectal thermistor probe inserted to 2.5 cm. Rectal temperatures were also measured following drug administration. The temperature difference between pre- and post-treatment values was calculated for each animal. In some experiments, mice received intravenous injections of SR141716A or its vehicle, 1 : 1 : 18 (emulphor-ethanol-saline), 10 min before O-1057. In these experiments, SR141716A was administered at a dose (3 mg kg^{-1}) known to antagonize *in vivo* effects of Δ^9-THC in mice (Compton *et al*., 1996) and O-1057 at a dose (0.1 mg kg^{-1} i.v.) producing approximately 80% of its maximal effects on spontaneous activity, nociception and rectal temperature (data not shown).

Analysis of data

Values have been expressed as means and variability as s.e.mean or as 95% confidence limits. Mean values have been compared using Student's unpaired *t*-test or analysis of variance followed by Dunnett's test or the Newman–Keuls test. A P value < 0.05 was considered to be significant. Effects of test compounds on forskolin-stimulated cyclic AMP production have been expressed in percentage terms. This was calculated from the equation $[100 \times (f' - b)]/(f - b)$ where f′, f

and b are values of cyclic AMP production (pmol ml^{-1}), f′ in the presence of forskolin and the test compound, f in the presence of forskolin only and b in the absence of both forskolin and the test compound (Ross *et al*., 1999). For *in vitro* experiments, values for EC_{50}, IC_{50} and maximal effects (E_{max}) and the s.e.mean or 95% confidence limits of these values have been calculated by non-linear regression analysis using the equation for a sigmoid concentration-response curve (GraphPad Prism). In organ bath experiments, the degree of drug-induced inhibition of evoked contractions has been expressed in percentage terms. This was calculated by comparing the amplitude of the twitch response after each addition of a twitch inhibitor with its amplitude immediately before the first addition of the inhibitor.

The K_B value of SR141716A for antagonism of cannabinoid in the vas deferens was calculated by substituting a single concentration ratio value into the equation $(x-1)=B/K_B$, where x (the 'concentration ratio') is the concentration of cannabinoid that produced a particular size of effect in the presence of SR141716A at a concentration, B, divided by the concentration of cannabinoid that produced an identical effect in the absence of SR141716A (Tallarida *et al*., 1979). Values of the concentration ratio and its 95% confidence limits were determined by symmetrical (2+2) dose parallel line assays (Colquhoun, 1971). This method was also used to establish whether the log concentration-response curves of cannabinoid in the presence and absence of SR141716A deviated significantly from parallelism.

For production of hypomotility and hypothermia, the data have been expressed as total number of photocell beam interruptions and rectal temperature change respectively. Antinociception was calculated as described above. ED_{50} values were determined from least-squares linear regression analysis followed by calculation of 95% confidence limits (Bliss, 1967; Wiley *et al*., 1998).

Drugs

CP55940 {(−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol} was supplied by Pfizer, Δ^9-THC by the National Institute on Drug Abuse and SR141716A {N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride} by Sanofi Recherche (*in vitro* experiments) or by the National Institute on Drug Abuse. O-1057 (Figure 1) was synthesized in the laboratory of Dr Razdan. Its maximum solubility in water was approximately 20 mg ml^{-1}. [^{3}H]-CP55940, 126 Ci $mmol^{-1}$ (CHO cell membranes) or 180 Ci $mmol^{-1}$ (rat brain membranes), was obtained from NEN Life Science Products.

Results

Effects of cannabinoids on [^{3}H]-CP55940 binding and on forskolin-stimulated cyclic AMP production

As shown in Figure 2, O-1057 readily displaced [^{3}H]-CP55940 from specific binding sites on both CB_1 and CB_2 CHO cell membranes with pK_i values of 8.36 and 7.95 respectively ($n=6$). These values were not significantly different (unpaired *t*-test). O-1057 also displaced [^{3}H]-CP55940 from specific binding sites on rat brain membranes with a pK_i value of

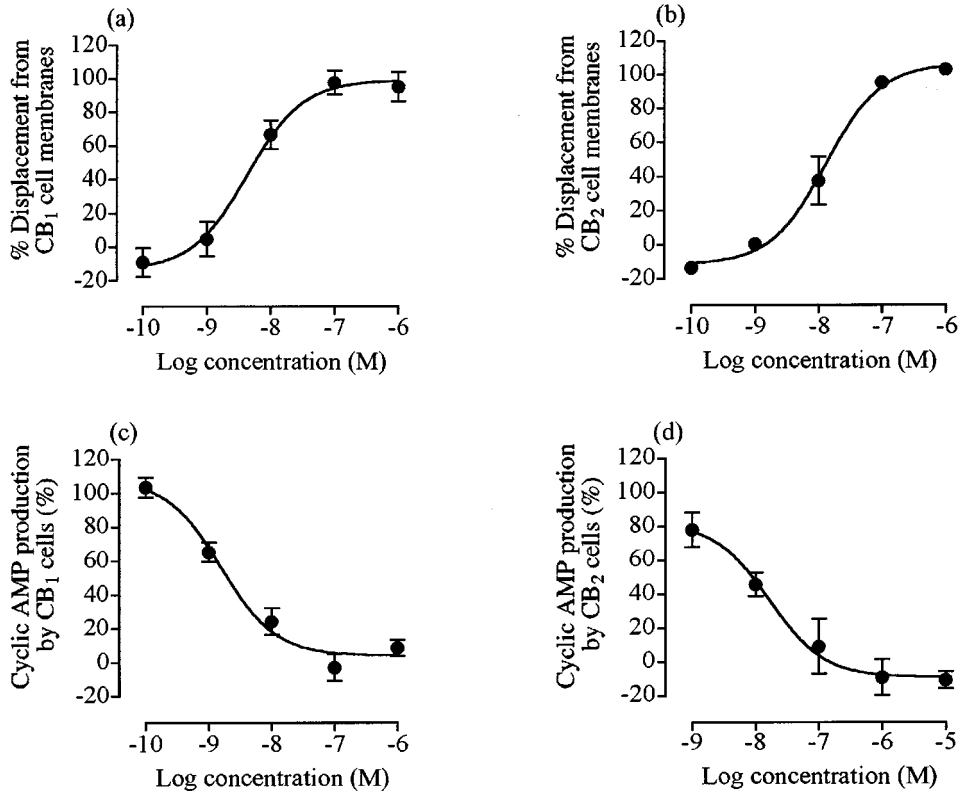

Figure 2 Upper panels: displacement of [^{3}H]-CP55940 (0.5 nM) by O-1057 (a) in CB_1- and (b) in CB_2-transfected CHO cells. Each symbol represents the mean per cent displacement ± s.e.mean ($n=6$). Lower panels: inhibition of forskolin-stimulated cyclic AMP production by O-1057 (c) in CB_1- and (d) in CB_2-transfected CHO cells. Each symbol represents mean percentage change in forskolin-stimulated cyclic AMP production ± s.e.mean ($n=4$ or 5). Forskolin-stimulated cyclic AMP production in the absence of O-1057 has been normalized to 100%.

Table 1 Effects on forskolin-stimulated cyclic AMP production by CB_1- and CB_2-transfected CHO cells

Cell line	*Cannabinoid*	*pEC_{50}*	*E_{max} (%)*
CB_1	O-1057	8.80 ± 0.17[a]	96.8 ± 4.3 (4)
	Δ^9-THC	8.04 ± 0.29[b]	107.5 ± 10.8 (4)
	CP55940	9.27 ± 0.17[a]	98.2 ± 8.7 (6)
CB_2	O-1057	7.78 ± 0.26	109.0 ± 6.8 (5)
	Δ^9-THC	7.68 ± 0.33	84.4 ± 10.6 (4)
	CP55940	8.59 ± 0.13	101.9 ± 5.2 (4)

[a]CB_1 and CB_2 pEC_{50} values are significantly different (unpaired *t*-test; $P = < 0.001$). [b]pEC_{50} (CB_1) significantly less than that of O-1057 or CP55940 (analysis of variance followed by the Newman–Keuls test; $P < 0.01$). The CP55940 data are from Ross *et al.* (1999) and were obtained using methods identical to those described in this paper.

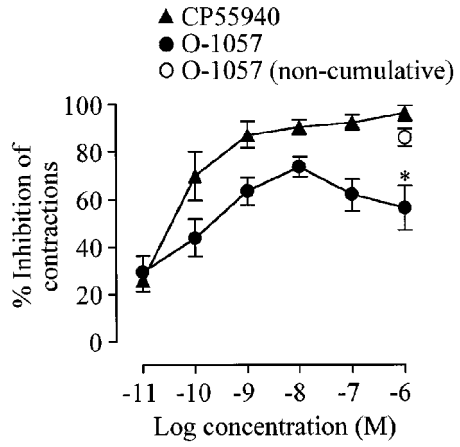

Figure 3 Mean log concentration response curves for CP55940 and O-1057 in the mouse isolated vas deferens. Each symbol represents the mean value ± s.e.mean for inhibition of electrically-evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition to the organ bath of CP55940 ($n = 6$) or O-1057 ($n = 12$). The asterisk indicates that the mean response to 1 μM O-1057 was significantly less ($P < 0.01$; unpaired *t*-test) when this was added to complete the construction of the cumulative log concentration-response curve shown than when it was added to tissues not previously exposed to O-1057 (non-cumulative; $n = 6$). The mean response to 1 μM CP55940 was 96.23 ± 0.41% when this was added to complete the construction of the cumulative log concentration-response curve shown and 95.25 ± 1.31% ($n = 6$) in tissues not previously exposed to CP55940 (not shown in Figure). These values are not significantly different (unpaired *t*-test).

7.86 ± 0.14 ($n = 3$). This value did not deviate significantly (analysis of variance followed by the Newman–Keuls test) from the corresponding pK_i value for Δ^9-THC in brain membranes (7.80 ± 0.15; $n = 3$) or from the pK_i of O-1057 in CB_1 CHO cell membranes (see above). O-1057 also shared the ability of the established cannabinoid receptor ligands, Δ^9-THC and CP55940, to inhibit cyclic AMP production in both CHO cell lines. In CB_1 transfected cells, the potency of O-1057 did not deviate significantly from that of CP55940 (Table 1). However, both these cannabinoids were more potent that Δ^9-THC. On the other hand, in CB_2 transfected cells, all three cannabinoids showed similar potencies as inhibitors of cyclic AMP production (Table 1). Like CP55940, O-1057 was significantly more potent as a CB_1 agonist than as a CB_2 agonist in the transfected cells. In untransfected CHO cells, O-1057 did not affect forskolin-stimulated cyclic AMP production at 1 or 10 μM (1-sample *t*-test; $n = 4$; data not shown). Nor did CP55940 (0.1–10 μM) inhibit cyclic AMP production in CB_2-transfected cells when a dispersion of CP55940 in water was used instead of the usual ethanolic stock solution (1-sample *t*-test; $n = 4$; data not shown).

Effects of CP55940 and O-1057 on electrically-evoked contractions of the mouse vas deferens

The concentration-response curve of CP55940 was sigmoid in shape, yielding pEC_{50} and E_{max} values of 10.53 ± 0.11 and 92.91 ± 2.94% respectively ($n = 6$) (Figure 3). In the range 0.01 to 10 nM, O-1057 also produced concentration-related decreases in the amplitude of evoked contractions of the vas deferens (Figure 3). Values of pEC_{50} and E_{max} calculated from the data obtained using this concentration range were 9.73 ± 0.59 and 76.84 ± 16.18% respectively ($n = 12$). The inhibitory effect of 10 nM O-1057 on electrically-evoked contractions could not be reversed by repeatedly draining and then replenishing organ baths with O-1057-free Krebs solution ($n = 6$; data not shown).

The degree of inhibition evoked by 100 nM or 1 μM O-1057 seemed to be less than that evoked by 10 nM. Although these apparent differences were not statistically significant (analysis of variance followed by Dunnett's test), the response to 1 μM O-1057 was significantly greater when this was added to tissues not previously exposed to O-1057 than when it was added to complete the construction of the cumulative log concentration-response curve shown in Figure 3. Because of this finding, we decided that we would investigate the ability of SR141716A to

Table 2 Effect of 100 nM SR141716A on inhibition of electrically-evoked contractions of the mouse vas deferens induced by O-1057 and CP55940

Pretreatment	*Agonist*	*Agonist concentration* (nM)	*Inhibition %*	*Potency ratio*	*pK_B*
Tween 80	O-1057	0.01	29.93 ± 4.29	80.9	8.90 ± 0.20
Tween 80	O-1057	0.1	71.02 ± 7.31	(30.0 & 202.7)	
SR141716A	O-1057	1.0	38.16 ± 6.05		
SR141716A	O-1057	10	69.46 ± 9.72		
Tween 80	CP55940	0.001	7.91 ± 1.49	94.9	8.97 ± 0.14
Tween 80	CP55940	0.01	50.20 ± 7.41	(48.3 & 184.9)	
SR141716A	CP55940	0.1	13.03 ± 2.11		
SR141716A	CP55940	1.0	46.79 ± 6.37		

Mean values with 95% confidence limits shown in brackets or s.e.mean ($n = 6$). Potency ratios indicate the dextral shifts produced by SR141716A in 2-point log concentration-response regression lines for O-1057 and CP55940, constructed using the agonist concentrations shown. These dextral shifts did not deviate significantly from parallelism. Potency ratios, confidence limits and deviations of pairs of log concentration-response regression lines from parallelism have been determined from symmetrical (2 + 2) dose parallel line assays (Colquhoun, 1971). Each pK_B value has been calculated from the corrresponding potency ratio value by Schild analysis. The pK_B values of SR141716A against O-1057 and CP55940 are not significantly different (unpaired *t*-test).

Table 3 *In vivo* effects of O-1057 and Δ^9-tetrahydrocannabinol in mice

Cannabinoid	*Route*	*ED_{50} hypokinesia* (mg kg^{-1})	*ED_{50} hypothermia* (mg kg^{-1})	*ED_{50} antinociception* (mg kg^{-1})
O-1057	i.v.	0.02 (0.01 & 0.09)	0.06 (0.05 & 0.07)	0.02 (0.01 & 0.03)
Δ^9-THC	i.v.	0.64 (0.60 & 0.68)	1.08 (0.77 & 1.50)	1.07 (0.77 & 1.47)
O-1057	p.o.	16.4 (10.1 & 26.4)	6.8 (5.1 & 9.0)	6.3 (4.3 & 9.0)
Δ^9-THC	p.o.	–	–	>100[a]
				(μg mouse^{-1})
O-1057	i.t.	–	–	4.79 (3.58 & 6.42)
Δ^9-THC	i.t.	–	–	31.0 (20.0 & 48.0)
O-1057	i.c.v.	–	–	1.80 (1.43 & 2.28)
Δ^9-THC	i.c.v.	–	–	32.1 (19.1 & 42.2)

Mean values with 95% confidence limits shown in brackets ($n=6$ or 12). [a]At an oral dose of 100 mg kg^{-1}, Δ^9-THC produced a mean score of 36.7% MPE in the tail flick test.

Table 4 Antagonism of O-1057 by SR141716A in mice

Pretreatment	*Treatment*	*Hypokinesia*[a]	*Hypothermia* (°C)	*Antinociception* (% MPE)	n
Vehicle	Vehicle	1606±226	0.1±0.2	5±4	6
Vehicle	O-1057	190±280[b]	−4.8±0.4[c]	85±15[c]	5
SR141716A	O-1057	903±369[d]	−0.7±0.5[d]	47±8[b]	6

Mean values with s.e.mean. SR141716A (3 mg kg^{-1} i.v.) was administered 10 min before O-1057 (0.1 mg kg^{-1} i.v.). [a]Total number of photocell beam interruptions over a 10 min period. [b]Significantly different from the vehicle/vehicle control value ($P<0.05$ for hypokinesia and $P<0.01$ for antinociception. [c]Significantly different from the other values in this column ($P<0.001$ for hypothermia and $P<0.05$ for antinociception). [d]Vehicle/vehicle control and SR141716A/O-1057 values were not significantly different ($P>0.05$). All comparisons were made using analysis of variance followed by the Newman–Keuls test.

attenuate O-1057-induced inhibition of evoked contractions by constructing 2-point log concentration-response plots in the absence or presence of this CB_1-selective antagonist making no more than one addition of O-1057 to any one tissue. As shown in Table 2, 100 nM SR141716A produced a significant dextral shift in the resulting 2-point log concentration-response plot that did not deviate significantly from parallelism. The pK_B value of SR141716A against CP55940 was also determined by constructing 2-point log concentration-response plots in the absence or presence of this antagonist. This value was not significantly different from the pK_B value of SR141716A against O-1057 (Table 2).

In vivo *effects of O-1057 and Δ^9-THC in mice*

When given intravenously, O-1057 was significantly more potent than Δ^9-THC in reducing spontaneous activity, decreasing rectal temperature and inducing antinociception in the tail flick test (Table 3). It was also more potent than Δ^9-THC in the tail flick test when injected intracerebroventricularly or intrathecally or when administered by gavage (Table 3). The ability of O-1057 to produce significant reductions in spontaneous activity, rectal temperature and nociception was abolished or significantly attenuated by intravenous pretreatment with SR141716A at a dose of 3 mg kg^{-1} (Table 4).

Discussion

The results obtained from our experiments with CHO cells transfected with human cannabinoid receptors show that O-1057, in aqueous solution, has high affinity for CB_1 and CB_2 receptors and behaves as an agonist at both these receptor types. At CB_1 receptors, the potency of O-1057 for inhibition of forskolin-stimulated cyclic AMP production was found not to deviate significantly from that of the potent cannabinoid receptor agonist, CP55940 (Pertwee, 1999). O-1057 also resembles CP55940 in showing slightly greater potency at CB_1 than at CB_2 receptors, the CB_2/CB_1 EC_{50} ratios of these two compounds for inhibition of cyclic AMP production being respectively 10.5 and 4.7. Additional support for our conclusion that O-1057 is an agonist for CB_1 receptors comes from the experiments we performed with the mouse isolated vas deferens. These showed first, that like other CB_1 receptor agonists (Pertwee, 1997), O-1057 inhibits electrically-evoked contractions of the mouse isolated vas deferens, second that this effect is antagonized by the selective, competitive, surmountable CB_1 receptor antagonist, SR141716A (Rinaldi-Carmona *et al*., 1994), and third, that the pK_B value of SR141716A against O-1057 (8.90) is almost the same as its pK_B value against the established CB_1 receptor agonist, CP55940 (8.97) and close to reported pK_D values of SR141716A for CB_1 receptors (8.91 to 9.72) obtained in saturation binding assays (Pertwee, 1999).

O-1057 also behaved as a CB_1 receptor agonist in our *in vivo* experiments with mice. Thus, as is to be expected for a CB_1 receptor agonist, it reduced spontaneous activity, lowered rectal temperature and exhibited antinociceptive activity in the tail flick test, showing a similar intravenous potency in all three tests (Martin *et al*., 1995). We also found that the ability of O-1057 to elicit these responses could be attenuated or abolished by pretreatment with SR141716A. The potency of intravenous O-1057 exceeded that of Δ^9-THC in these bioassays by 32, 18 and 53.5-fold respectively (Table 3). O-1057 was also more potent than Δ^9-THC in the tail flick test when administered orally, intrathecally or intracerebroventricularly. These data are consistent with the results obtained in our experiments with CB_1-transfected cells which showed O-1057 to be significantly more potent than Δ^9-THC as an inhibitor of forskolin-stimulated cyclic AMP production. The findings that, in

aqueous solution, O-1057 shows cannabimimetic activity *in vivo*, that it is active intravenously and orally, and that it exhibits greater antinociceptive potency than Δ^9-THC by these routes, strengthens the case for investigating the therapeutic potential of this water-soluble cannabinoid in man.

Vasa deferentia were more sensitive to 1 μM O-1057 when they had not been exposed previously to this agent than when they had already been subjected to a series of increasing concentrations of O-1057 during the construction of a cumulative concentration-response curve. One possible explanation for this finding is that prior exposure to O-1057 can cause desensitization. In line with this hypothesis are reports firstly, that *in vitro* administration of WIN55212-2 can induce tolerance to its inhibitory effect on electrically-evoked contractions of the myenteric plexus preparation of guinea-pig small intestine (Basilico *et al*., 1999) and secondly, that *in vivo* administration of Δ^9-THC can render mouse vasa deferentia tolerant to inhibition of evoked contractions induced by *in vitro* administration of this or other cannabinoids (Pertwee *et al*., 1993; Pertwee & Griffin, 1995). Whether this hypothesis is valid remains to be established. Also requiring further investigation is the question of why, in spite of its solubility in water, we could not reverse the inhibitory effect of O-1057 on vasa deferentia by repeatedly washing the tissues with cannabinoid-free Krebs solution. In the meantime, it remains possible that this apparent irreversibility is an indication that O-1057 is a pro-drug that elicits responses only after its hydrolysis to another, more lipophilic compound.

In conclusion, this paper describes some pharmacological properties of a water-soluble cannabinoid ester, O-1057, that exhibits cannabimimetic properties both *in vitro* and *in vivo*. Our *in vitro* data showed it to be a potent agonist at both CB_1 and CB_2 receptors. The *in vivo* data provide further evidence for the ability of O-1057 to activate CB_1 receptors and also indicate that it would be worth mounting a clinical trial with O-1057 to explore its clinical potential as an analgesic using oral or intravenous administration or delivery by aerosol. Further experiments are now required to establish whether or not O-1057 is a pro-drug that is converted to a more active species by hydrolysis. It will also be important to determine whether other water-soluble cannabinoid receptor ligands, including antagonists, can be synthesized using a similar chemical strategy to the one we adopted for O-1057.

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