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RESEARCH PAPER

Cannabidiol displays unexpectedly high potency as an antagonist of CB_1 and CB_2 receptor agonists *in vitro*

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Background and purpose: A nonpsychoactive constituent of the cannabis plant, cannabidiol has been demonstrated to have low affinity for both cannabinoid CB_1 and CB_2 receptors. We have shown previously that cannabidiol can enhance electrically evoked contractions of the mouse vas deferens, suggestive of inverse agonism. We have also shown that cannabidiol can antagonize cannabinoid receptor agonists in this tissue with a greater potency than we would expect from its poor affinity for cannabinoid receptors. This study aimed to investigate whether these properties of cannabidiol extend to CB_1 receptors expressed in mouse brain and to human CB_2 receptors that have been transfected into CHO cells.
Experimental approach: The [^{35}S]GTPγS binding assay was used to determine both the efficacy of cannabidiol and the ability of cannabidiol to antagonize cannabinoid receptor agonists (CP55940 and *R*-(+)-WIN55212) at the mouse CB_1 and the human CB_2 receptor.
Key results: This paper reports firstly that cannabidiol displays inverse agonism at the human CB_2 receptor. Secondly, we demonstrate that cannabidiol is a high potency antagonist of cannabinoid receptor agonists in mouse brain and in membranes from CHO cells transfected with human CB_2 receptors.
Conclusions and implications: This study has provided the first evidence that cannabidiol can display CB_2 receptor inverse agonism, an action that appears to be responsible for its antagonism of CP55940 at the human CB_2 receptor. The ability of cannabidiol to behave as a CB_2 receptor inverse agonist may contribute to its documented anti-inflammatory properties.
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Keywords: cannabidiol; CP55940; O-2654; inverse agonism; neutral antagonism; GTPγS assay; CB_1 receptor; CB_2 receptor; mouse brain

Abbreviations: CI, confidence intervals; CP55940, (–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; Δ^9-THC, Δ^9-tetrahydrocannabinol; DMEM, Dubbecco's modified Eagle's medium; DMSO, dimethyl sulphoxide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; FBS, fetal bovine serum; hCB_1-CHO, Chinese hamster ovary cells stably transfected with human CB_1 receptors; hCB_2-CHO, Chinese hamster ovary cells stably transfected with human CB_2 receptors; *R*-(+)-WIN55212, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone; SR144528, *N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo [2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide

Introduction

Cannabis sativa is now known to contain at least 70 compounds that are unique to it and known collectively as cannabinoids (ElSohly and Slade, 2005). One of these is (–)-Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive constituent of cannabis, and another is (–)-cannabidiol, which is not psychoactive and exhibits much lower affinity than Δ^9-THC for cannabinoid CB_1 and CB_2 receptors (Showalter *et al*., 1996; Thomas *et al*., 1998; Pertwee, 1999; Bisogno *et al*., 2001; Thomas *et al*., 2004). Cannabidiol is of interest because it lacks psychoactivity and yet has therapeutic potential, for example for the management of inflammation, anxiety, emesis and nausea, and as a neuroprotective agent (Pertwee, 2004). Indeed, together with

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Δ^9-THC, cannabidiol is a major constituent of Sativex, a medicine that is now licensed in Canada for neuropathic pain associated with multiple sclerosis.

In previous experiments (Pertwee *et al*., 2002), cannabidiol was found to share the ability of the CB_1-selective inverse agonist/antagonist, rimonabant, to increase the amplitude of electrically evoked contractions of the mouse vas deferens (Pertwee *et al*., 2002), which, for rimonabant at least, is most likely an indication of inverse agonist activity (Pertwee, 2005). Cannabidiol was also found to resemble rimonabant in its ability to antagonize (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (*R*-(+)-WIN55212)- and (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol (CP55940)-induced inhibition of electrically evoked contractions of the mouse vas deferens in a competitive, surmountable manner (Pertwee *et al*., 1995, 2002). Unlike rimonabant, however, cannabidiol produced this antagonism at concentrations well below those at which it binds to CB_1 (or CB_2) cannabinoid receptors, suggesting that it was competing with *R*-(+)-WIN55212 and CP55940 for an as yet uncharacterized non-CB_1 pharmacological target on nerve terminals. These properties of cannabidiol prompted this current study.

Thus, the present investigation was directed primarily at investigating whether the unexpectedly high potency exhibited by cannabidiol as an antagonist of cannabinoid receptor agonists in the mouse vas deferens extends to cannabinoid receptors in mouse brain tissue and/or to Chinese hamster ovary cells stably transfected with human CB_2 receptors (hCB_2-CHO) cell membranes. Cannabidiol was compared with rimonabant in the brain tissue experiments and with *N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528), an established CB_2 receptor inverse agonist/antagonist in the experiments performed with hCB_2-CHO cell membranes. We also addressed the question of whether cannabidiol behaves as an inverse agonist or as a neutral antagonist at CB_1 and/or CB_2 receptors. Accordingly, in some experiments cannabidiol was compared with a putative neutral cannabinoid receptor antagonist, the synthetic cannabidiol analogue, O-2654 (Thomas *et al*., 2004). This compound differs from cannabidiol and rimonabant by behaving as a neutral antagonist of cannabinoid receptor agonists rather than as an inverse agonist in the mouse isolated vas deferens (Pertwee, 2005).

In this study, we report first that cannabidiol can behave as an inverse agonist at the human CB_2 receptor. Second, we demonstrate that cannabidiol behaves as a high-potency antagonist of cannabinoid receptor agonists in mouse brain tissue and in membranes from CHO cells transfected with human CB_2 receptors. Furthermore, the high potency of cannabidiol as an antagonist of the cannabinoid receptor agonist CP55940 in the hCB_2-CHO cell membranes appears to be a consequence of the ability of cannabidiol to behave as an inverse agonist at the hCB_2 receptor. Some of the results described in this paper have been presented to the International Cannabinoid Research Society (Thomas *et al*., 2006).

Materials and methods

The methods used comply with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines for the use of experimental animals.

Animals

MF1 mice were purchased from Harlan UK Ltd (Blackthorn, UK), whereas C57BL/6 CB_1 receptor knockout mice and the wild-type (WT) littermates were obtained from NIH (Rockville, MD, USA). Mice were maintained on a 12/12 h light/dark cycle with free access to food and water.

CHO cells

CHO cells stably transfected with cDNA encoding human cannabinoid CB_2 receptors were maintained in Dulbecco's modified Eagles's medium (DMEM) nutrient mixture F-12 HAM, supplemented with 2 mM L-glutamine, 10% fetal bovine serum (FBS), 0.6% penicillin–streptomycin, hygromycin B (300 $\mu g\,ml^{-1}$) and geneticin (600 $\mu g\,ml^{-1}$). The CHO cells stably transfected with cDNA-encoding human cannabinoid CB_1 receptors ($B_{max} = 2980 \pm 802$ fmol mg^{-1} protein) were maintained in DMEM F-12 supplemented with 10% FBS, geneticin (400 $\mu g\,ml^{-1}$) and zeocin (250 $\mu g\,ml^{-1}$). The native CHO cells were maintained in DMEM nutrient mixture F-12 HAM, which was supplemented with 2 mM L-glutamine, 5% FBS and 2% penicillin–streptomycin. All cells were maintained at 37°C and 5% CO_2 in their respective media and were passaged twice a week using non-enzymatic cell dissociation solution.

Membrane preparation

Binding assays with [^{3}H]CP55940 and with [^{35}S]GTPγS were performed with mouse whole-brain membranes, prepared as described by Thomas *et al*. (2004), or with native CHO cell membranes, or with membranes from CHO cells transfected with either human CB_1 or CB_2 receptors (Ross *et al*., 1999). The buffers used in the preparation of [^{35}S]GTPγS-binding assay brain membranes were additionally supplemented with 100 mM $NaCl_2$.

The CHO cells were removed from flasks by scraping and then frozen as a pellet at −20°C until required. The CB_1-CHO cells were additionally FBS-starved for 24 h before their removal from flasks. Before use in a radioligand-binding assay, cells were defrosted, diluted in 50 mM Tris buffer (radioligand displacement assay) or GTPγS-binding buffer ([^{35}S]GTPγS-binding assay) and homogenized with a 1 ml hand-held homogenizer. Protein assays were performed using a Bio-Rad Dc kit (Bio-Rad, Hercules, CA, USA).

Radioligand displacement assay

The assays were carried out with [^{3}H]CP55940, 1 mg ml^{-1} bovine serum albumin (BSA) and 50 mM Tris buffer, total assay volume 500 μl, using the filtration procedure described previously (Ross *et al*., 1999; Thomas *et al*., 2005). Binding was initiated by the addition of either the brain membranes

(33 μg protein per tube) or the hCB_2-CHO cells (25 μg protein per tube) and all assays were performed at 37°C for 60 min. Specific binding was defined as the difference between the binding that occurred in the presence and the absence of 1 μM unlabelled CP55940. The concentration of [^{3}H]CP55940 used in the displacement assays was 0.7 nM. All drugs were stored as a stock solution of 10 mM in dimethyl sulphoxide (DMSO), the vehicle concentration in all assay tubes being 0.1% DMSO. The binding parameters for [^{3}H]CP55940, determined by fitting data from saturation-binding experiments to a one-site saturation plot using GraphPad Prism, were 2336 ± 878 fmol mg^{-1} protein (B_{max}) and 2.31 ± 1.33 nM (K_d) in mouse brain membranes and 72418 ± 4279 fmol mg^{-1} protein (B_{max}) and 1.04 ± 0.14 nM (K_d) in hCB_2-CHO cells (Thomas *et al*., 2005).

[^{35}S]GTPγS-binding assay

The method used for measuring agonist-stimulated [^{35}S]GTPγS-binding to CB_1 and to human CB_2 receptors was as described previously by Thomas *et al*. (2005). The GTPγS-binding buffer contained 50 mM Tris-HCl, 50 mM Tris base, 5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, 1 mM dithiothreitol and 0.1% BSA. Briefly, the assay conditions for experiments conducted in mouse brain membranes included 30 μM GDP, 10 μg ml^{-1} protein and 0.1 nM [^{35}S]GTPγS, in a final volume of 500 μl. The corresponding assay conditions for experiments conducted in hCB_2-CHO cell membranes were 320 μM GDP, 10 μg ml^{-1} protein and 0.7 nM [^{35}S]GTPγS, in a final volume of 250 μl. Experiments conducted in native CHO cells were performed under identical conditions to those used for experiments with hCB_2-CHO cell membranes, whereas the conditions used for the CB_1-CHO cells were similar to those used for experiments conducted with mouse brain membranes. The only difference from the mouse brain experimental conditions was that the NaCl in the GTPγS buffer was omitted. Additionally, CB_1-CHO cells were 24 h FBS-starved, unlike the CB_2-CHO and native CHO cells. Non-specific binding was measured in the presence of 30 μM GTPγS and the drugs were incubated in the assay for 60 min at 30°C. Additionally, mouse brain membranes were preincubated for 30 min at 30°C with 0.5 U ml^{-1} adenosine deaminase (200 U mg^{-1}) to remove endogenous adenosine. All drugs, with the exception of morphine, were stored as a stock solution of 1 or 10 mM in DMSO. The vehicle concentration in experiments conducted using one of these drugs was 0.1% DMSO or 0.11% DMSO in the presence of an antagonist. In experiments with morphine, which was stored as a stock solution of 10 mM in distilled water, we used a vehicle concentration of 0.01% DMSO.

Analysis of data

Values have been expressed as means and variability as s.e.m. or as 95% confidence intervals (CI). The concentration of a drug that produced a 50% displacement of [^{3}H]CP55940 from specific binding sites (IC_{50}) was calculated using GraphPad Prism 4. Its dissociation constant (K_i) was calculated using the equation of Cheng and Prusoff (1973). Net ligand-stimulated [^{35}S]GTPγS-binding values were calculated by subtracting basal binding values (obtained in the absence of ligand) from ligand-stimulated values (obtained in the presence of ligand) as detailed elsewhere (Ross *et al*., 1999). These values were compared with the level of basal binding by performing a one-sample *t*-test (GraphPad Prism). A *P*-value <0.05 was considered to be significant. Values for EC_{50}, for maximal effect (E_{max}) and for the s.e.m. or 95% confidence limits of these values have been calculated by nonlinear regression analysis using the equation for a sigmoid concentration–response curve (GraphPad Prism).

The apparent dissociation constant (K_B) values for antagonism of agonists by cannabidiol, rimonabant, SR144528 or O-2654 have been calculated by Schild analysis from the concentration ratio, defined as the concentration of an agonist that elicits a response of a particular size in the presence of a competitive reversible antagonist at a concentration, B, divided by the concentration of the same agonist that produces an identical response in the absence of the antagonist. The methods used to determine concentration ratios and apparent K_B values and to establish whether log concentration–response plots deviated significantly from parallelism are detailed elsewhere (Pertwee *et al*., 2002). Mean values have been compared using Student's two-tailed *t*-test for unpaired data or one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test (GraphPad Prism). A *P*-value <0.05 was considered to be significant.

Our experiments were further analysed to determine the effect of cannabidiol or SR144528 on CP55940-stimulated [^{35}S]GTPγS binding to hCB_2-CHO cell membranes or to determine the effect of cannabidiol or rimonabant on CP55940-stimulated [^{35}S]GTPγS binding to mouse brain membranes. Thus, we subtracted the inhibitory effect that cannabidiol, SR144528 or rimonabant induced on the basal level of [^{35}S]GTPγS binding, determined in the absence of any other compound, from the effect obtained in the presence of CP55940 and re-calculated the apparent K_B values in the same manner as described above.

Materials

Cannabidiol was supplied by GW Pharmaceuticals (Porton Down, Wiltshire, UK), THC by the National Institute on Drug Abuse (Bethesda, MD, USA) and O-2654 by Dr Raj Razdan (Organix Inc., MA, USA). Rimonabant and SR144528 were obtained from Sanofi-Aventis (Montpellier, France). CP55940, *R*-(+)-WIN55212 and morphine were from Tocris (Bristol, UK) and BSA, TRIZMA hydrochloride, TRIZMA base, L-glutamine, penicillin–streptomycin, non-enzymatic cell dissociation solution, guanosine 5′-diphosphate (GDP) and 8-cyclopentyl-1,3-dipropylxantine (DPCPX) from Sigma-Aldrich (St Louis, MO, USA). [^{3}H]CP55940 (160 Ci mmol^{-1}) was obtained from APBiotech (Little Chalfont, UK) and [^{35}S]GTPγS (1250 Ci mmol^{-1}) from PerkinElmer (Boston, MA, USA). GTPγS, adenosine deaminase and hygromycin B from Roche Diagnostic (Indianapolis, IN, USA) and the geneticin from Gibco (Paisley, UK).

Results

Experiments with mouse brain membranes

Initially, we compared the abilities of rimonabant and cannabidiol to antagonize CP55940-induced stimulation of [^{35}S]GTPγS binding to mouse brain membranes. At 1 μM, cannabidiol shared the ability of 10 nM rimonabant to produce a rightward shift in the log concentration–response curve of CP55940 (Figure 1a and b). Both the apparent K_B values of rimonabant (0.09 nM) and cannabidiol (79 nM) for the antagonism of CP55940-induced stimulation of [^{35}S]GTPγS binding to mouse brain were well below their corresponding CB_1 K_i values (2.2 nM and 4.9 μM, respectively, see also Table 1). Similar apparent K_B values were obtained for the antagonism of *R*-(+)-WIN55212-mediated [^{35}S]GTPγS binding by either 1 μM cannabidiol or 10 nM rimonabant to mouse brain membranes (Figure 2). The apparent K_B values of cannabidiol or rimonabant, with 95% CI in parantheses, were 138 nM (87 and 225 nM) and 0.3 nM (0.16 and 0.52 nM), respectively, for this antagonism of *R*-(+)-WIN55212 in mouse brain membranes.

Next, we confirmed that under our laboratory assay conditions, it was possible to detect not only stimulation of [^{35}S]GTPγS binding to mouse brain membranes by the established CB_1 receptor agonists, CP55940 and *R*-(+)-WIN55212, but also inhibition of such binding by a CB_1 receptor inverse agonist, rimonabant (Figure 3, Table 2). We then went on to investigate the effect of cannabidiol by itself on [^{35}S]GTPγS binding to mouse brain membranes. Although this compound had no detectable effect at 1 or 100 nM, it produced significant inhibition at 1 and 10 μM (Figure 3a). The inhibitory effect produced by 1 μM cannabidiol did not deviate significantly from that of 1 μM rimonabant, whereas the inhibitory effect of 10 μM cannabidiol greatly exceeded that of 10 μM rimonabant ($P<0.05$; ANOVA followed by Bonferroni's multiple comparison test; $n=6$ and 8).

Our next experiments were performed with the putative neutral CB_1 receptor antagonist, O-2654 (Thomas *et al.*, 2004), to establish how it compared with cannabidiol as a modulator of [^{35}S]GTPγS binding to mouse brain membranes. In contrast to CP55940, *R*-(+)-WIN55212, cannabidiol or rimonabant, O-2654 neither inhibited nor enhanced [^{35}S]GTPγS binding between 0.1 nM and 1 μM to mouse brain membranes (Figure 3b). Unexpectedly, at a concentration of 10 μM, O-2654 significantly inhibited the binding of [^{35}S]GTPγS to mouse brain membranes by an amount that

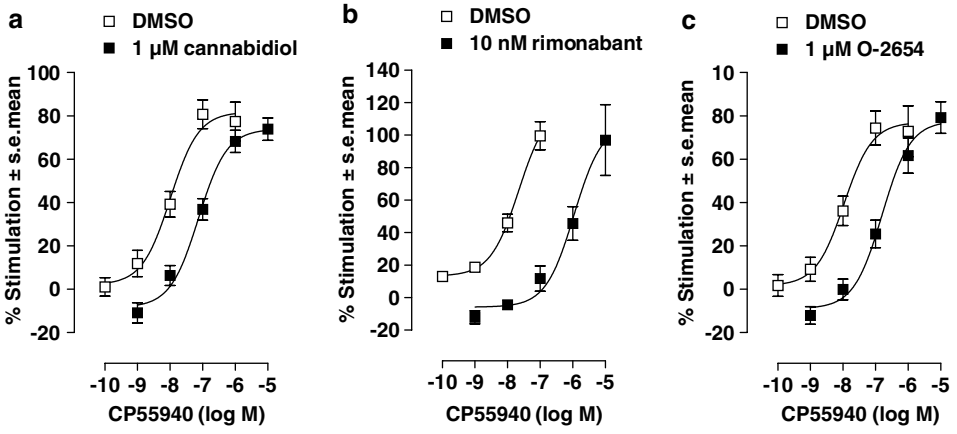

Figure 1 [^{35}S]GTPγS binding to mouse brain membranes. The effect of (**a**) 1 μM cannabidiol ($n=4$–5), (**b**) 10 nM rimonabant ($n=4$) or (**c**) 1 μM O-2654 ($n=5$) on the mean log concentration–response curve of CP55940 for stimulation of [^{35}S]GTPγS binding to mouse whole-brain membranes. Each symbol represents the mean percentage increase in [^{35}S]GTPγS binding ± s.e.m. Mean apparent K_B values of these cannabinoids for their antagonism of CP55940 have been calculated from these data and are listed in Table 1.

Table 1 The mean apparent K_B values for antagonism of CP55940-induced [^{35}S]GTPγS binding to membranes and the K_i values for the displacement of [^{3}H]CP55940 from membranes

Antagonist	*Membrane preparation*	*Mean apparent K_B (95% CI)*	n	*K_i for displacement of [^{3}H]CP55940 (95% CI)*	n
Rimonabant (10 nM)	Mouse brain	0.09 nM (0.02 and 0.41 nM)	4[a]	2.2 nM (1.1 and 4.2 nM)	6
Cannabidiol (1 μM)		79 nM (48 and 128 nM)	5	4.9 μM (2.1 and 11.3 μM)	5–12[b]
O-2654 (1 μM)		51 nM (34 and 74 nM)	5	114 nM (96 and 137 nM)	5–11[b]
SR144528 (100 nM)	hCB$_2$-CHO cell	0.49 nM (0.26 and 0.85 nM)	6[a]	7.5 nM (5.8 and 9.8 nM)	6–8
Cannabidiol (1 μM)		65 nM (16 and 228 nM)	5	4.2 μM (2.9 and 6.2 μM)	6–8
O-2654 (1 μM)		30 nM (12 and 59 nM)	5	48 nM (30 and 76 nM)	6–8

Abbreviations: CI, confidence intervals; CP55940, (–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; hCB$_2$-CHO, Chinese hamster ovary cells stably transfected with human CB_2 receptors; SR144528, *N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide.

[a]Data previously published in Thomas *et al.* (2005).

[b]Data previously published in Thomas *et al.* (2004).

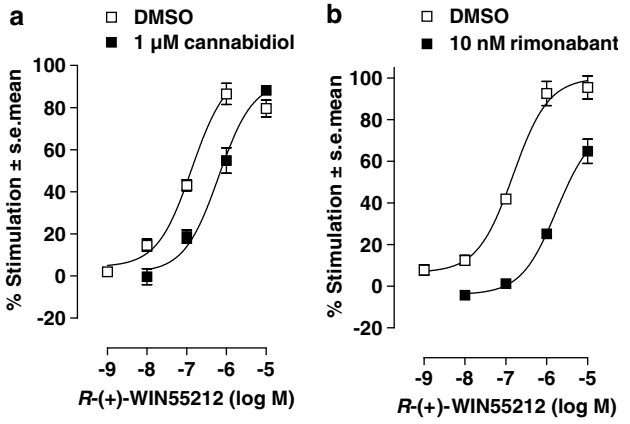

Figure 2 [^{35}S]GTPγS binding to mouse brain membranes. The effect of (**a**) 1 μM cannabidiol ($n=6$) or (**b**) 10 nM rimonabant ($n=5$) on the mean log concentration–response curve of *R*-(+)-WIN55212 for stimulation of [^{35}S]GTPγS binding to mouse whole-brain membranes. Each symbol represents the mean percentage increase in [^{35}S]GTPγS binding ± s.e.m. The mean apparent K_B values of cannabidiol or rimonabant for their antagonism of *R*-(+)-WIN55212, with 95% CI in parantheses were 0.3 nM (0.16 and 0.52 nM) and 138 nM (87 and 225 nM), respectively.

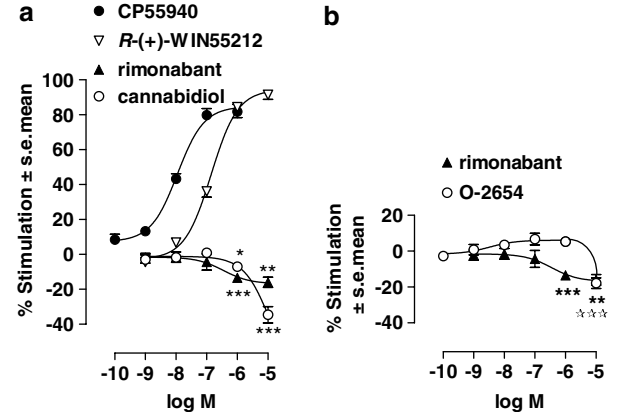

Figure 3 [^{35}S]GTPγS binding to mouse brain membranes. The effect of (**a**) CP55940 ($n=9$), *R*-(+)-WIN55212 ($n=7$–8), rimonabant ($n=7$–8) and cannabidiol ($n=6$) or (**b**) rimonabant ($n=7$–8) and O-2654 ($n=6$–10) on the level of [^{35}S]GTPγS binding to mouse whole-brain membranes. Each symbol represents the mean percentage change in [^{35}S]GTPγS binding ± s.e.m. In (**a**), asterisks (*) denote rimonabant and cannabidiol values and in (**b**), asterisks (*) or open stars (☆) denote rimonabant or O-2654 values, respectively, which are significantly different from zero (*$P<0.05$, **$P<0.005$, ***$P<0.001$; one-sample *t*-test). The mean EC_{50} and E_{max} values of these cannabinoids are listed in Table 2.

did not differ significantly from the inhibition produced by 10 μM rimonabant ($P>0.05$; ANOVA followed by Bonferroni's multiple comparison test; $n=8$). We also found that O-2654 shared the ability of cannabidiol to antagonize CP55940 (Figure 1c). However, unlike cannabidiol and rimonabant, O-2654 is only slightly more potent as a CB_1 receptor antagonist (apparent $K_B=51$ nM; Table 1) than as a CB_1 receptor ligand ($K_i=114$ nM) (Thomas *et al*., 2004). The K_i values of rimonabant, cannabidiol and O-2654 and their mean apparent K_B values for antagonism of CP55940-induced stimulation of [^{35}S]GTPγS binding to mouse brain membranes are shown in Table 1.

Because cannabidiol produced an inverse effect at 10 μM in mouse brain membranes, which was so much greater than that produced by rimonabant, we investigated whether this effect of cannabidiol is CB_1 receptor-mediated. This issue was addressed by establishing whether membranes prepared from the brains of mice whose CB_1 receptors had been genetically deleted ($CB_1^{-/-}$ C57BL/6 mice) responded differently, in the [^{35}S]GTPγS assay, to cannabidiol from brain membranes obtained from their WT littermates.

In these experiments, 10 μM cannabidiol was no less effective as an inhibitor of [^{35}S]GTPγS binding to $CB_1^{-/-}$ than to WT C57BL/6 mouse brain membranes (Figure 4a). It is noteworthy, however, that cannabidiol was less potent at inhibiting [^{35}S]GTPγS binding to WT C57BL/6 mouse brain membranes than to the MF1 mouse brain membranes that were used in all our other experiments. Thus, 1 μM cannabidiol produced detectable inhibition only in the MF1 mouse brain membranes (Figures 3a and 4a). Rimonabant was also less potent in this assay when it was conducted with WT C57BL/6 rather than MF1 mouse brain membranes. Thus, like cannabidiol, 1 μM rimonabant inhibited [^{35}S]GTPγS binding only to the MF1 mouse brain membranes (Figures 3 and 4b). Neither 1 nor 10 μM rimonabant inhibited [^{35}S]GTPγS binding to $CB_1^{-/-}$ mouse brain membranes (Figure 4b). This finding was unexpected, as Breivogel *et al*. (2001) have reported that rimonabant can inhibit [^{35}S]GTPγS binding to brain membranes obtained from C57BL/6 $CB_1^{-/-}$ mice.

Cannabidiol and rimonabant exhibited lower inhibitory potency in the [^{35}S]GTPγS-binding assay when this was performed with brain membranes obtained from C57BL/6 mice rather than from MF1 mice. Consequently, as we had already found O-2654 to possess at least 10 times less inhibitory potency than either cannabidiol or rimonabant in this assay when it was performed with MF1 mouse brain membranes, and as O-2654 inhibited [^{35}S]GTPγS binding to these membranes at 10 μM but not 1 μM, we did not investigate the effect of O-2654 on [^{35}S]GTPγS binding to C57BL/6 mouse brain membranes.

Because the results from our experiments with $CB_1^{-/-}$ mouse brain membranes suggest that cannabidiol can inhibit [^{35}S]GTPγS binding through a CB_1 receptor-independent mechanism, we performed experiments with membranes prepared either from CHO cells transfected with human CB_1 receptors or from untransfected CHO cell membranes. We found, however, that although binding of [^{35}S]GTPγS to the hCB_1-CHO cell membranes was stimulated by cannabidiol at concentrations between 1 and 1000 nM and inhibited by cannabidiol at 10 μM (Figure 5), none of these concentrations of cannabidiol affected [^{35}S]GTPγS binding to the membranes obtained from untransfected CHO cells (data not shown).

We also investigated whether cannabidiol would antagonize ligand-induced activation of a non-cannabinoid G protein-coupled receptor. More specifically, we addressed the question of whether cannabidiol can block the activation of opioid receptors by morphine as, like the CB_1 receptor, opioid receptors are thought to signal primarily through $G_{i/o}$ proteins (Corbett *et al*., 2006). We selected morphine for these experiments as it is expected to target all the opioid

Table 2 The mean E_{max} values ± s.e.m. and the mean EC_{50} values, with 95% CI in parentheses for the efficacy of tested cannabinoids in mouse brain or hCB_2-CHO cell membranes

Cannabinoid	*Membrane preparation*	E_{max} *% stimulation above basal*	*EC_{50} (95% confidence intervals)*	n
CP55940	Mouse brain	85 ± 3	11 nM (7 and 17 nM)	9
R-(+)-WIN55212		94 ± 3	140 nM (94 and 209 nM)	7–8
Rimonabant		−17 ± 3	351 nM (32 and 3901 nM)	7–8
Cannabidiol		ND	ND	
O-2654		ND	ND	
CP55940	hCB_2-CHO cell	43 ± 3	0.5 nM (0.2 and 1.9 nM)	6–12
SR144528		−16 ± 1	0.8 nM (0.2 and 2.4 nM)	5
Cannabidiol		−15 ± 5	503 nM (22 nM and 11 μm)	8
O-2654		−17 ± 3	240 nM (37 and 1570 nM)	6

Abbreviations: CI, confidence intervals; hCB_2-CHO, Chinese hamster ovary cells stably transfected with human CB_2 receptors; ND, not defined; *R*-(+)-WIN55212, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone; SR144528, *N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo [2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide.

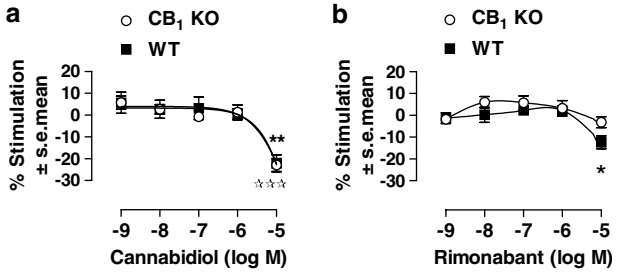

Figure 4 [^{35}S]GTPγS binding to mouse brain membranes. The effect of (**a**) cannabidiol ($n = 5$–6) or (**b**) rimonabant ($n = 6$) on the level of [^{35}S]GTPγS binding to mouse whole-brain membranes prepared from either C57BL/6 mice whose CB_1 receptors had been genetically deleted (open circles) or their WT littermates (filled squares). Values represent the mean percentage inhibition of [^{35}S]GTPγS binding ± s.e.m.. Asterisks (*) or open stars (☆) denote values obtained from WT or CB_1 KO membranes, respectively, which are significantly different from zero (*$P < 0.01$, **$P < 0.005$, ***$P < 0.001$; one-sample *t*-test). The response induced by 10 μM rimonabant in mouse brain membranes prepared from $CB_1^{-/-}$ mice differed significantly to that induced in mouse brain membranes prepared from the WT littermates (*$P < 0.05$; Student's *t*-test; $n = 6$).

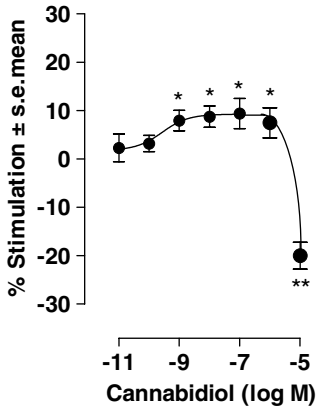

Figure 5 [^{35}S]GTPγS binding to hCB_1-CHO cell membranes. The effect of cannabidiol ($n = 5$–15) on the level of [^{35}S]GTPγS binding to CB_1 transfected CHO cell membranes. Each symbol represents the mean percentage increase in [^{35}S]GTPγS binding ± s.e.m.. Asterisks (*) denote values obtained, which are significantly different from zero (*$P < 0.05$, **$P < 0.001$; one-sample *t*-test).

receptor subtypes that are thought to be present in mouse brain membranes (Mignat *et al*., 1995). We found that 1 μM cannabidiol ($n = 4$–6) did not significantly antagonize morphine-induced enhancement of [^{35}S]GTPγS binding to mouse brain membranes (data not shown).

Experiments with cannabidiol and SR144528 using human CB_2-CHO cell membranes

Having established the effect of cannabidiol on CB_1 receptor-containing systems (mouse brain and CB_1-CHO cell membranes), we compared the abilities of SR144528 and cannabidiol to displace [^{3}H]CP55940 from hCB_2-CHO cell membranes. The results (Figure 6) provided the CB_2 K_i values for SR144528 and cannabidiol shown in Table 1. We next investigated whether cannabidiol shares the ability of SR144528 to antagonize CP55940-induced stimulation of [^{35}S]GTPγS binding to hCB_2-CHO cell membranes. At a concentration of 1 μM, cannabidiol produced a downward as well as a rightward shift of the log concentration–response curve of CP55940 (Figure 7a) and its apparent K_B value was calculated to be 64.5 times less than its CB_2 K_i value (Table 1). Similar results were obtained from experiments with SR144528 performed under the same assay conditions (Figure 7b). Thus, SR144528 induced a downward as well as rightward displacement of the CP55940 log concentration–response curve and exhibited an apparent K_B value that was 15 times less than its CB_2 K_i value (Table 1).

We also investigated whether cannabidiol shares the ability of SR144528 to behave as a CB_2 receptor inverse agonist, as measured by inhibition of [^{35}S]GTPγS binding to hCB_2-CHO cell membranes. Cannabidiol was indeed found to produce an inhibitory effect on [^{35}S]GTPγS binding, as was SR144528 (Figure 8a). The E_{max} of cannabidiol did not deviate significantly from that of SR144528, whereas its pEC_{50} (6.3 ± 0.7) was markedly greater than that of SR144528 (9.1 ± 0.3). A summary of these results can be found in Table 2.

Experiments with O-2654 using human CB_2-CHO cell membranes

As O-2654 attenuated CP55940 responses in mouse brain membranes in a manner that is consistent with it being

a neutral CB_1 receptor antagonist, it was of interest to determine whether O-2654 also behaves as a neutral antagonist at the CB_2 receptor. Accordingly, we first tested how well O-2654 displaces [^{3}H]CP55940 from the hCB_2-CHO cell membranes (Figure 6), the results obtained indicating that O-2654 binds 2.4 times more readily to the CB_2 receptor than to the CB_1 receptor (Table 1). We then addressed the question of whether O-2654 resembles cannabidiol (and SR144528) in antagonizing CP55940-induced inhibition of [^{35}S]GTPγS binding more potently than it displaces [^{3}H]CP55940 from hCB_2-CHO cell membranes. Our experiments showed that this was not so, as the apparent K_B value of O-2654 for antagonism of CP55940 (Figure 7c) was not markedly different from its CB_2 K_i value (Table 1). O-2654 appeared to induce downward as well as rightward displacements of the CP55940 log concentration–response curve in the hCB_2-CHO cell membranes in these experiments (Figure 7c). However, in contrast to our findings with cannabidiol and SR144528, this downward displacement was not statistically significant. Thus, the 95% CI for the bottom of the CP55940 log concentration–response curves in the absence or presence of 1 μM O-2654 overlapped. Although, we also discovered that when added by itself, O-2654 exhibits cannabidiol-like potency and efficacy as an inhibitor of [^{35}S]GTPγS binding to hCB_2-CHO cell membranes (Table 2 and Figure 8b), this should not be taken as evidence that O-2654 is a CB_2 receptor inverse agonist. Thus, the potency and efficacy of O-2654 as an inhibitor of [^{35}S]GTPγS binding remained the same irrespective of whether the bioassay was performed with hCB_2-CHO cell membranes or with membranes from cells that had not been transfected with CB_2 receptors ($n=6$; Figure 9). In contrast, CP55940 ($n=6$), cannabidiol ($n=8$), or SR144528 ($n=8$) did not modulate [^{35}S]GTPγS binding to membranes prepared from CHO cells that had not been transfected with CB_2 receptors (data not shown).

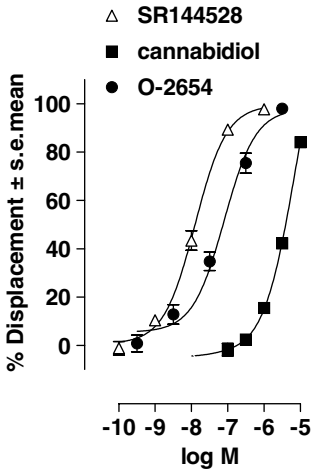

Figure 6 Displacement of [^{3}H]CP55940 from hCB_2-CHO cell membranes. The ability of SR144528, cannabidiol or O-2654 to displace [^{3}H]CP55940 from specific binding sites in hCB_2-CHO cell membranes. Each symbol represents the mean percent displacement ± s.e.m. The mean K_i values for this displacement were calculated by the Cheng–Prusoff equation and are listed in Table 1.

Finally, it is unlikely that cannabidiol and SR144528 each appear to be more potent as an antagonist of CP55940-induced stimulation of [^{35}S]GTPγS binding to hCB_2-CHO cell membranes than as a displacer of [^{3}H]CP55940 from CB_2 receptors because the buffers used in the [^{35}S]GTPγS and [^{3}H]CP55940-binding assays were different. Thus, the ability of SR144528 ($n=5$; data not shown) to displace [^{3}H]CP55940 from hCB_2-CHO cell membranes was unaffected when GTPγS buffer was used in this bioassay instead of the standard Tris/BSA buffer. We have also reported similar findings previously with Δ^9-tetrahydrocannabivarin (Thomas *et al.*, 2005).

A summary of the CB_2 K_i values of cannabidiol, SR144528 and O-2654 and of the mean apparent K_B values of these compounds for antagonism of CP55940-induced stimulation of [^{35}S]GTPγS binding to hCB_2-CHO cell membranes can be found in Table 1.

Discussion

The results described in this paper indicate that the unexpectedly high potency reported previously for cannabi-

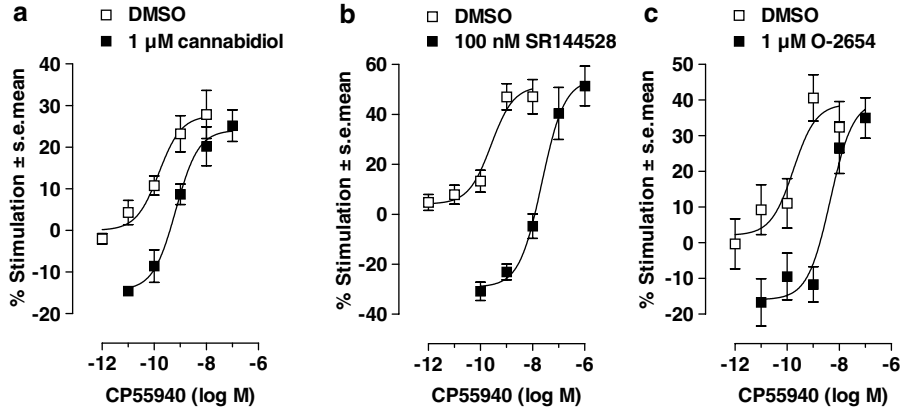

Figure 7 [^{35}S]GTPγS binding to membranes from hCB_2-CHO cell membranes. The effect of (**a**) 1 μM cannabidiol ($n=4$–5), (**b**) 100 nM SR144528 ($n=5$–6) or (**c**) 1 μM O-2654 ($n=3$–5) on the mean log concentration–response curve of CP55940 for stimulation of [^{35}S]GTPγS binding to CB_2-transfected CHO cell membranes. Each symbol represents the mean percentage increase in [^{35}S]GTPγS binding ± s.e.m. Mean apparent K_B values of these cannabinoids for their antagonism of CP55940 have been calculated from these data and are listed in Table 1.

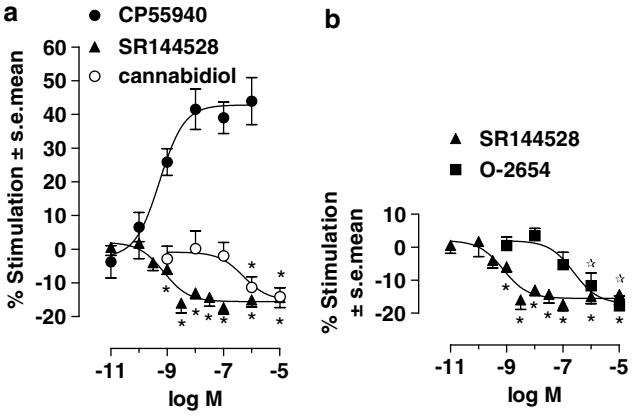

Figure 8 [^{35}S]GTPγS binding to membranes from hCB$_2$-CHO cell membranes. The effect of (**a**) CP55940 ($n=6$–12), SR144528 ($n=5$) and cannabidiol ($n=8$) or (**b**) SR144528 ($n=5$) and O-2654 ($n=6$) on the level of [^{35}S]GTPγS binding to CB$_2$ transfected CHO cell membranes. Each symbol represents the mean percentage change in [^{35}S]GTPγS binding ± s.e.m. In (**a**), asterisks (*) denote SR144528 and cannabidiol values and in (**b**), asterisks (*) or open stars (☆) denote SR144528 or O-2654 values, respectively, which are significantly different from zero (*$P<0.05$; one-sample *t*-test). The mean EC_{50} and E_{max} values of these cannabinoids are listed in Table 2.

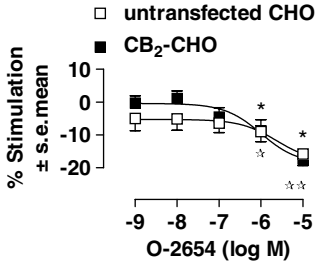

Figure 9 [^{35}S]GTPγS binding to membranes from untransfected cells or cells transfected with human CB$_2$ receptors. The effect of O-2654 on the level of [^{35}S]GTPγS binding to untransfected CHO cell membranes ($n=6$) or CB$_2$-transfected CHO cell membranes ($n=8$). Each symbol represents the mean percentage change in [^{35}S]GTPγS binding ± s.e.m. Asterisks (*) or open stars (☆) denote values obtained from CB$_2$-transfected CHO cell membranes or untransfected cell membranes, respectively, which are significantly different from zero (*$P<0.05$, **$P<0.01$; one-sample *t*-test).

diol-induced antagonism of cannabinoid agonists in the mouse vas deferens (Pertwee *et al*., 2002) extends to the brain. The apparent K_B values for the antagonism of CP55940 or *R*-(+)-WIN55212 are at least 35 times lower than the K_i values of cannabidiol for displacement of [^{3}H]CP55940 from mouse brain membranes (Showalter *et al*., 1996; Thomas *et al*., 1998, 2004; Bisogno *et al*., 2001 see also Table 1). However, they are similar to the corresponding apparent K_B values (34.0 and 120.3 nM, respectively) obtained for cannabidiol in the mouse vas deferens (Pertwee *et al*., 2002), suggesting that this cannabinoid may be acting on the same target in the brain as in the vas deferens. Cannabidiol appears to exhibit at least some selectivity as an antagonist of CP55940 and *R*-(+)-WIN55212, since 1 μM cannabidiol did not antagonize stimulation of [^{35}S]GTPγS binding to mouse brain membranes induced by the μ-, δ- and κ-opioid receptor agonist, morphine (Mignat *et al*., 1995). We have also found in a previous investigation (Pertwee *et al*., 2002) that cannabidiol is markedly less potent as an antagonist of DAMGO, a selective μ-opioid receptor agonist, than as an antagonist of *R*-(+)-WIN55212 or CP55940 in the mouse vas deferens. Although, cannabidiol has been reported to modulate allosterically μ- and δ-opioid receptors (Kathmann *et al*., 2006), this occurs only at high micromolar concentrations and it is therefore unlikely that this interaction occurred in our experiments.

Rimonabant also exhibited greater potency as an antagonist of CP55940- and *R*-(+)-WIN55212-induced stimulation of [^{35}S]GTPγS binding to mouse brain membranes than as a CB$_1$ receptor ligand. Thus, the apparent K_B values of rimonabant for antagonism of these two cannabinoid receptor agonists were respectively 24 and 7 times lower than the K_i of rimonabant for its displacement of [^{3}H]CP55940 from mouse brain membranes. Interestingly, such a K_i/K_B discrepancy has not been detected in the mouse-isolated vas deferens (Pertwee *et al*., 1995). This may be because rimonabant exhibits greater potency as an antagonist of CP55940 and *R*-(+)-WIN55212 in brain tissue than in the vas deferens because first, *R*-(+)-WIN55212 and CP55940 inhibit electrically evoked contractions of this tissue not only by acting through CB$_1$ receptors but also by activating non-CB$_1$ targets (see Pertwee *et al*., 2002, 2005; Thomas *et al*., 2005) and because these putative non-CB$_1$ targets exhibit little or no sensitivity to antagonism by rimonabant.

By themselves, cannabidiol and rimonabant both inhibited [^{35}S]GTPγS binding to mouse brain membranes. Cannabidiol exhibited particularly high inverse agonist efficacy, producing inhibition of [^{35}S]GTPγS binding at 10 μM, which greatly exceeded that produced by 10 μM rimonabant. Interestingly, in experiments using assay conditions almost identical to those used in the present investigation, Breivogel *et al*. (2001) found that cannabidiol (concentration unspecified) did not produce any significant effects on [^{35}S]GTPγS binding to C57BL/6 mouse brain membranes. On the other hand, 10 μM cannabidiol has been reported to inhibit [^{35}S]GTPγS binding to rat cerebellar membranes (Petitet *et al*., 1998).

The results from our experiments with membranes prepared from CB$_1$-transfected and -untransfected CHO cells suggest that cannabidiol can inhibit [^{35}S]GTPγS binding by interacting with the CB$_1$ receptor as an inverse agonist at 10 μM. However, since cannabidiol-inhibited [^{35}S]GTPγS binding to membranes obtained from mice whose CB$_1$ receptors had been genetically deleted as well as from WT mice, it is likely that this cannabinoid can also inhibit [^{35}S]GTPγS-binding through one or more CB$_1$ receptor-independent mechanisms. This in turn raises the possibility that the inverse effect of 10 μM cannabidiol on MF1 mouse brain membranes greatly exceeded that of 10 μM rimonabant (Figure 3a) because cannabidiol was interacting with more than one pharmacological target in an additive or synergistic manner. That cannabidiol and rimonabant exhibited lower potency as inhibitors of [^{35}S]GTPγS binding to brain membranes when these were obtained from C57BL/6 mice

rather than from MF1 mice may be because one or more of their targets was more highly expressed by the MF1 mice.

One question raised is whether cannabidiol was inhibiting [^{35}S]GTPγS binding to mouse brain membranes because, similar to rimonabant, it can block adenosine A_1 receptors when these are being activated by endogenously released adenosine (Savinainen *et al*., 2003). Thus, Savinainen *et al*. (2003) have found that at a concentration of 1 μM, the selective A_1 receptor antagonist DPCPX prevents rimonabant from inhibiting [^{35}S]GTPγS binding to rat cerebellar membranes. Moreover, cannabidiol has recently been found to inhibit the cellular uptake of adenosine (Carrier *et al*., 2006), an effect that would be expected to augment any inverse effect arising from A_1 receptor blockade. It is unlikely, however, that cannabidiol inhibited [^{35}S]GTPγS binding to brain membranes in the present investigation by acting through A_1 receptors. Thus, we have found that 1 μM DPCPX does not alter the ability of 100 nM, 1 or 10 μM cannabidiol to inhibit [^{35}S]GTPγS binding to mouse brain membranes ($n=3$; data not shown). Moreover the experiments in which Breivogel *et al*. (2001) found cannabidiol not to inhibit [^{35}S]GTPγS binding to $CB_1^{+/+}$ mouse brain membranes (see above) were performed in the absence of any A_1 receptor antagonist and in the presence of much less exogenously added adenosine deaminase (0.004 U ml^{-1}) than in our experiments (0.5 U ml^{-1}).

As in mouse brain membranes, in experiments with hCB_2-CHO cell membranes, cannabidiol was also found to act more potently as an antagonist of CP55940-induced stimulation of [^{35}S]GTPγS binding than would be expected from its ability to displace [^{3}H]CP55940 from hCB_2-CHO cell membranes. Similar results were obtained with SR144528. SR144528 has been reported previously to behave as an inverse agonist at the CB_2 receptor (Bouaboula *et al*., 1999; Portier *et al*., 1999; Ross *et al*., 1999; Rhee and Kim, 2002), and this was confirmed by the results obtained in the present study with hCB_2-CHO cell membranes. Cannabidiol also behaved as a CB_2 receptor inverse agonist as it shared the ability of SR144528 to induce an inhibition of [^{35}S]GTPγS binding to hCB_2-CHO cell membranes when added by itself.

There is evidence from the results obtained in this investigation that this antagonism of CP55940 by 1 μM cannabidiol in the hCB_2-CHO cell membrane experiments may have been non-competitive in nature. Thus, 1 μM cannabidiol produced a marked downward displacement of the CP55940 log concentration–response curve for stimulation of [^{35}S]GTPγS binding to hCB_2 receptors (Figure 7a) and re-analysis of these data in a manner expected to exclude the effect of cannabidiol by itself (see above) suggests that this downward displacement accounts entirely for the antagonism of CP55940 induced by 1 μM cannabidiol in the hCB_2-CHO cell membrane experiments (Figure 10a). In terms of the two-state model (Leff, 1995), it may be that CP55940 stimulates [^{35}S]GTPγS binding to CB_2 receptors by shifting the equilibrium between constitutively active (R*) and inactive (R) receptors more towards R*, whereas cannabidiol shifts this equilibrium towards R, thereby 'physiologically' opposing the ability of CP55940 to stimulate CB_2 receptors. Hence at 1 μM, a concentration at which it induces little displacement of [^{3}H]CP55940 from hCB_2 receptors (Figure 6),

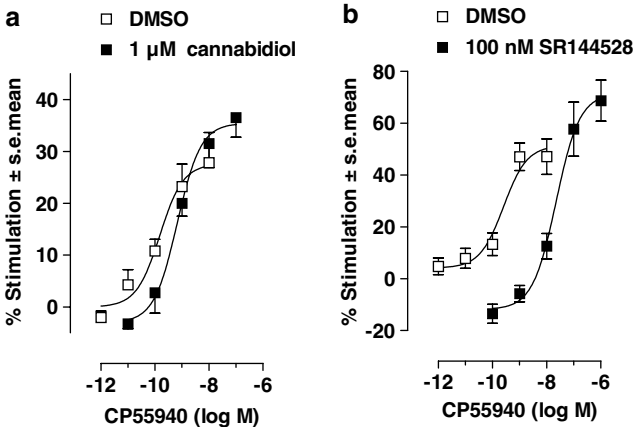

Figure 10 [^{35}S]GTPγS binding to membranes from hCB_2-CHO cell membranes. The effect of (**a**) 1 μM cannabidiol ($n=4$–5) or (**b**) 100 nM SR144528 ($n=5$–6) on the mean log concentration–response curve of CP55940 for stimulation of [^{35}S]GTPγS binding to CB_2-transfected CHO cell membranes after subtraction of the inhibitory effect induced by either 1 μM cannabidiol or 100 nM SR144528 at the basal level of [^{35}S]GTPγS binding, determined in the absence of any other compound. Each symbol represents the mean percentage increase in [^{35}S]GTPγS binding $\pm$ s.e.m. After this re-analysis, it was found that 1 μM cannabidiol did not produce a significant rightward shift of the CP55940 log concentration–response curve, whereas 100 nM SR144528 antagonized CP55940 with an apparent K_B value of 2.5 nM, with 95% CI of 1.6 and 4.3 nM.

cannabidiol may have been antagonizing CP55940 at the CB_2 receptors entirely through inverse agonism and not at all by direct competition with CP55940 for receptors in the R* state.

As to the antagonism of CP55940 induced by 100 nM SR144528 at the CB_2 receptor, this may have been partly competitive in nature and partly a result of inverse agonism. Thus, when the component of SR144528-induced antagonism of CP55940 that seemingly arises from its ability to inhibit [^{35}S]GTPγS binding to hCB_2-CHO cell membranes was excluded, a significant SR144528-induced rightward shift in the log concentration–response curve of CP55940 was still apparent (Figure 10b). Although, there still appears to be a downward displacement of the CP55940 log concentration–response curve, this was not found to be statistically significant. Thus, the 95% CI for the bottom of the CP55940 log concentration–response curves in the absence or presence of 100 nM SR144528 overlapped. The apparent K_B value of SR144528 calculated from this shift is much closer to the CB_2 K_i value of SR144528 than the corresponding apparent K_B value calculated from the data shown in Figure 7b, however, this recalculated K_B value of SR144528 remains significantly less than its CB_2 K_i value. It is possible, therefore, that the E_{max} of SR144528 for inhibiting [^{35}S]GTPγS binding to hCB_2-CHO cell membranes underestimates its maximal inverse efficacy. This may be because an insufficient proportion of the hCB_2 receptors was constitutively active in the absence of CP55940, thereby making it possible for SR144528 to produce a further degree of inverse agonism in the presence of CP55940, which according to the two-state model would be expected to shift the equilibrium for CB_2 receptors from R to R* and so increase the number of CB_2 receptors in the putative constitutively active R* state (Leff, 1995). This hypothesis is supported by results obtained with O-2654. This ligand does not appear to

significantly inhibit [^{35}S]GTPγS binding to hCB$_2$-CHO cell membranes when administered by itself at 1 μM and it antagonized CP55940-induced stimulation of [^{35}S]GTPγS binding to hCB$_2$ receptors with an apparent K_B value that does not deviate significantly from its hCB$_2$ K_i value (Table 1). Further experiments will be required to test this hypothesis more fully and also to address the related question of whether the abilities of cannabidiol and rimonabant to behave as inverse agonists in mouse brain membranes accounts at least in part for our finding that these ligands antagonize CP55940-induced stimulation of [^{35}S]GTPγS binding to mouse brain membranes more potently than they displace [^{3}H]CP55940 from such membranes (Table 1).

In conclusion, this paper provides evidence that cannabidiol exhibits unexpectedly high potency *in vitro* as an antagonist of both CB$_1$ and CB$_2$ receptor agonists and that this antagonism is non-competitive in nature. The mechanism by which cannabidiol antagonized CB$_1$ receptor agonists in our experiments remains to be elucidated, one possibility being that it can also attenuate any responses induced by CP55940 or *R*-(+)-WIN55212 in brain membranes from CB$_1^{-/-}$ mice. It is noteworthy, however, that Breivogel *et al*. (2001) have reported that in contrast to *R*-(+)-WIN55212, CP55940 does not stimulate [^{35}S]GTPγS binding to such membranes. As to the high potency displayed by cannabidiol as an antagonist of CB$_2$ receptor activation, our data suggest that this may stem from its ability to induce CB$_2$ receptor inverse agonism at concentrations well below those at which it displaces [^{3}H]CP55940 from these receptors. This action may also contribute to the well-known anti-inflammatory properties of cannabidiol (reviewed in Pertwee, 2004), as there is evidence that CB$_2$ receptor inverse agonism can inhibit immune cell migration (Lunn *et al*., 2006). This paper also contains further evidence that O-2654 can behave as a neutral CB$_1$ receptor antagonist, at least at concentrations of up to 1 μM, whereas under the same assay conditions cannabidiol and the established inverse agonist, rimonabant, can behave as inverse agonists at concentrations of 1 and 10 μM. O-2654 may also be a neutral CB$_2$ receptor antagonist. Thus, although it inhibited [^{35}S]GTPγS binding to hCB$_2$-CHO cell membranes, it appeared to do so in a CB$_2$ receptor-independent manner.

Acknowledgements

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Conflict of interest

The authors state no conflict of interest.

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