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European Journal of Pharmacology 464 (2003) 207–215
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Pharmacological characterisation of cannabinoid receptors inhibiting interleukin 2 release from human peripheral blood mononuclear cells

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Received 5 September 2002; received in revised form 22 January 2003; accepted 28 January 2003

Abstract

The effects of a range of cannabinoid receptor agonists and antagonists on phytohaemagglutinin-induced secretion of interleukin-2 from human peripheral blood mononuclear cells were investigated. The nonselective cannabinoid receptor agonist WIN55212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3-[4-morpholinylmethyl]pyrrolo[1,2,3-de]1,4-benzoxazin-6-yl](1-naphthyl) methanone mesylate) and the selective cannabinoid CB_2 receptor agonist JWH 015 ((2-methyl-1-propyl-1*H*-indol-3-yl)-1-napthalenylmethanone) inhibited phytohaemagglutinin (10 μg/ml)-induced release of interleukin-2 in a concentration-dependent manner ($IC_{1/2max}$, WIN55212-2 = 8.8×10^{-7} M, 95% confidence limits (C.L.) = 2.2×10^{-7}–3.5×10^{-6} M; JWH 015 = 1.8×10^{-6} M, 95% C.L. = 1.2×10^{-6}–2.9×10^{-6} M, $n = 5$). The nonselective cannabinoid receptor agonists CP55,940 ((−)-3-[2-hydroxy-4-(1,1-dimethyl-hepthyl)-phenyl]4-[3-hydroxypropyl]cyclo-hexan-1-ol), Δ^9-tetrahydrocannabinol and the selective cannabinoid CB_1 receptor agonist ACEA (arachidonoyl-2-chloroethylamide) had no significant ($P > 0.05$) inhibitory effect on phytohaemagglutinin-induced release of interleukin-2. Dexamethasone significantly ($P < 0.05$) inhibited phytohaemagglutinin-induced release of interleukin-2 in a concentration-dependent manner ($IC_{1/2max} = 1.3 \times 10^{-8}$ M, 95% C.L. = 1.4×10^{-9}–3.2×10^{-8} M). The cannabinoid CB_1 receptor antagonist SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride) (10^{-6} M) did not antagonise the inhibitory effect of WIN55212-2 whereas the cannabinoid CB_2 receptor antagonist 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) antagonised the inhibitory effect of WIN55212-2 ($pA_2 = 6.3 \pm 0.1$, $n = 5$). In addition, CP55,940 (10^{-6} M) and Δ^9-tetrahydrocannabinol (10^{-6} M) also antagonised the inhibitory effects of WIN55212-2 ($pA_2 = 6.1 \pm 0.1$, $n = 5$ and $pA_2 = 6.9 \pm 0.2$, $n = 5$). In summary, WIN55,212-2 and JWH 015 inhibited interleukin-2 release from human peripheral blood mononuclear cells via the cannabinoid CB_2 receptor. In contrast, CP55,940 and Δ^9-tetrahydrocannabinol behaved as partial agonists/antagonists in these cells.

Keywords: Cannabinoid; Interleukin-2; Phytohaemagglutinin; Peripheral blood mononuclear cell

1. Introduction

Cannabinoids have been shown to downregulate the immune system (for reviews, see Cabral and Dove Pettit, 1998; Berdyshev, 2000). This conclusion is partly based on an early in vivo study by Morahan et al. (1979) who demonstrated a decreased resistance of mice to *Listeria monocytogens* or *Herpes simplex* virus infections after treatment with Δ^9-tetrahydrocannabinol. Consistent with these findings are a number of in vitro studies in which cannabinoids have been reported to inhibit T cell mitogenesis and interleukin-2 production from T lymphocyte cell lines (for reviews, see Klein et al., 1998a,b).

Interleukin-2 is an important cytokine responsible for T lymphocyte signalling during proliferation and macrophage/monocyte activation during inflammatory episodes (Herrman et al., 1989). The expression of functional interleukin-2 receptors is another variable that determines how long the clonal proliferation of T cells occurs after antigen stimulation (Smith, 1988). In general, interleukin-2 regulates both antigen-specific and non-antigen-specific proliferation of T cells, natural killer cells and B cells.

The discovery and cloning of two cannabinoid receptors, CB_1 and CB_2, has begun to give new clues as to how these drugs affect the immune system (Matsuda et al., 1990; Munro et al, 1993). Cannabinoid receptors are members of the G-protein-coupled receptor family (Bayewitch et al., 1995).

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doi:10.1016/S0014-2999(03)01379-7

While cannabinoid CB_1 receptors are found in the brain with low levels of expression in the peripheral tissues, cannabinoid CB_2 receptors are expressed primarily in immune tissues (Bouaboula et al., 1993; Galiegue et al., 1995; Kaminski et al., 1992), suggesting that the majority of the immunomodulatory properties of cannabinoids may be mediated via cannabinoid CB_2 receptors, although to date, very few studies have been reported to support this hypothesis.

The density of cannabinoid CB_2 receptors on immune cells is 10–100 times that of cannabinoid CB_1 receptors, as shown by semi-quantitative reverse transcription polymerase chain reaction and Northern blotting studies (Galiegue et al., 1995). The rank order of cannabinoid CB_2 receptor expression on human blood leukocytes is B cells>NK cells>monocytes>polymorphonuclear neutrophils>T8 cells>T4 cells (Parolaro, 1999). Furthermore, it has been shown that cannabinoid receptor expression in peripheral blood mononuclear cells is altered upon stimulation with phytohaemagglutinin (Daaka et al., 1996), suggesting an active role for the cannabinoid system in immune responses.

Given the proinflammatory properties of interleukin-2, modulation of its release via cannabinoid receptors would present an attractive pharmacological target for the treatment of various inflammatory conditions. In the present study, the effects of cannabinoid receptor ligands on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells have been investigated. A preliminary account of part of this report has been presented in abstract form to The International Cannabinoid Research Society (Ihenetu et al., 2002).

2. Materials and methods

2.1. Drugs and reagents

CP55,940 ((−)-3-[2-hydroxy-4-(1,1-dimethyl-heptyl)-phenyl]4-[3-hydroxypropyl]cyclo-hexan-1-ol) was a generous gift from Pfizer. SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride) and 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) were gifts from Sanofi Recherche (Montpellier, France). WIN55212-2 mesylate ((*R*)-(+)-[2,3-dihydro-5-methyl-3-[4-morpholinylmethyl]pyrrolo[1,2,3-de]1,4-benzoxazin-6-yl](1-naphthyl) methanone mesylate), ACEA (arachidonoyl-2-chloroethylamide) and JWH 015 ((2-methyl-1-propyl-1*H*-indol-3-yl)-1-napthalenylmethanone) were purchased from Tocris, Cookson (Bristol, UK). MTT (3-[4,5-dimethylthiazole-2-yl]2,5-diphenyl tetrazolium bromide) was purchased from Sigma-Aldrich (Dorset, UK). CP55,940, SR141716A, SR144528 and ACEA were dissolved in ethanol whereas WIN55,212-2 and JWH 015 were dissolved in dimethyl sulphoxide (DMSO) and stored at −20 °C at a concentration of 10 mM. Accordingly, these solvents were included in all assays at a final concentration of 0.1% as vehicle controls.

2.2. Preparation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells were isolated from buffy coat cells purchased from the National Blood Transfusion Service (NBTS) (Brentwood, Essex, UK). Separation of peripheral blood mononuclear cells was done by density gradient centrifugation using Histopaque R-1077 (Sigma-Aldrich), based on the modification of the original method described by Boyum (1968). In brief, buffy coat cells were diluted (1:2, v/v) with sterile phosphate-buffered saline and human peripheral blood mononuclear cells were isolated by density gradient centrifugation (2500 × *g* for 25 min) in an Accuspin tube (Sigma-Aldrich). Cells recovered from the interface between the plasma and Histopaque solution were washed twice in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (1700 × *g* for 10 min). Peripheral blood mononuclear cells were resuspended in RPMI-1640 medium supplemented with L-glutamine (2 mM), penicillin (50 U/ml) and streptomycin (50 μg/ml), and 10% heat-inactivated foetal calf serum. Aliquots were removed and cells were counted and assayed for viability by the trypan blue dye exclusion method and the MTT assay. Slides of the cell suspension were made, stained with a Romanowsky stain (May Grunwald-Giemsa) and a differential cell count obtained by examination of the slide under a microscope (magnification 1000 ×).

2.3. Interleukin-2 secretion

Human peripheral blood mononuclear cells were adjusted to a density of 1×10^6 cells/ml with RPMI-1640 medium and cultured in 24-well plates (Falcon, Becton Dickinson, Pont De Claire, France) in foetal calf serum-free RPMI-1640 medium, at 37 °C in a humidified atmosphere with 5% CO_2. Cells were preincubated with CP55940 (10^{-10}–10^{-5} M), WIN55212-2 (10^{-10}–10^{-5} M), Δ^9-tetrahydrocannabinol (10^{-10}–10^{-5} M), JWH 015 (10^{-10}–10^{-5} M) or dexamethasone (10^{-10}–10^{-6} M) for 2 h before stimulation with phytohaemagglutinin (10 μg/ml). Supernatants were harvested after 18 h incubation and stored at −70 °C until assayed for interleukin-2 by ELISA. In experiments where the effects of antagonists were studied, cells were preincubated with SR141716A (10^{-6} M), SR144528 (10^{-6} M), CP55940 (10^{-6} M) or Δ^9-tetrahydrocannabinol (10^{-6} M) for 30 min before the addition of the cannabinoid agonist or dexamethasone.

2.4. Enzyme-linked immunosorbent assay

Interleukin-2 release was measured by enzyme-linked immunosorbent assay (ELISA) of the culture supernatants according to the manufacturer's guidelines. In brief, anti-human interleukin-2 monoclonal capture antibody (Pharmingen B.D., Oxford, UK; cat. no. 555051) was paired with

biotinylated anti-human interleukin-2 monoclonal detection antibody (cat. no. 555040). Ninety-six-well plates (Nunc-immunoplates maxisorp F96, Pharmingen B.D.), were coated with 1 μg/ml capture antibody at 4 °C for 24 h. Following washing, blocking and addition of standards (10–2000 pg/ml) and samples (undiluted), a one-step detection comprising the use of biotinylated antibody/streptavidin-linked peroxidase (both 0.5 μg/ml), respectively, was carried out. Tetramethylammonium benzidine was used as a substrate solution and reaction was stopped with 2 M H_2SO_4 solution. Absorbance was read at a wavelength of 450 nm.

2.5. Statistical analysis

Concentration–effect curves were analysed by Prism (GraphPad, San Diego, CA, USA). Other results are shown as bar graphs. In some experiments, the results are expressed as percentage inhibition of interleukin-2 release from phytohaemagglutinin-treated cells. $IC_{1/2max}$ values were calculated by Prism and pA_2 values calculated from single agonist concentration–ratio values by the Schild equation assuming a slope of unity (Kenakin, 1993). All values are expressed as arithmetic (pA_2 values) or geometric mean ($IC_{1/2max}$ values) ± standard error of the mean (S.E.M.) or 95% confidence limit (C.L.) as appropriate. Statistical significance was determined using a one-sample *t*-test or analysis of variance followed by an appropriate post hoc test. Statistical significance was assumed if *P* value was ≤ 0.05.

3. Results

3.1. Purity and viability of human peripheral blood mononuclear cells

Human peripheral blood mononuclear cell preparations, prepared from buffy coat cells, comprised approximately 95% lymphocytes and 5% monocytes as measured by differential leukocyte counts. Furthermore, after 18 h incubation in serum-free medium, 99.17% ± 4.99% ($n=4$) of the lymphocytes were recovered from the medium.

Under our experimental conditions, the viability of human peripheral blood mononuclear cells isolated from buffy coat cells exceeded 95% on all occasions, when determined by trypan blue dye exclusion and by the MTT assay. This viability was not significantly ($P>0.05$) altered by incubation of human peripheral blood mononuclear cells for 18 h with phytohaemagglutinin, dexamethasone or any of the cannabinoid receptor ligands studied in foetal calf serum-free RPMI-1640 medium.

3.2. The effect of phytohaemagglutinin on interleukin-2 secretion from human peripheral blood mononuclear cells

Nonstimulated human peripheral blood mononuclear cells constitutively released minimal amounts of interleukin-2 (14 ± 10 pg/ml, $n=5$) after 18 h incubation at 37 °C (Fig. 8). Following stimulation with phytohaemagglutinin (10 μg/ml), a marked release of interleukin-2 was observed over 18 h (1869 ± 54 pg/ml, $n=5$, Fig. 1). Stimulation of human peripheral blood mononuclear cells with phytohaemagglutinin (10 μg/ml) evoked a minimal release of interleukin-2 within the first 6 h and a rise between 12 and 18 h. The peak release of interleukin-2 was seen at 18 h (Fig. 1). There was no significant change ($P>0.05$) in cell numbers between phytohaemagglutinin (10 μg/ml)-stimulated and nonstimulated cells over 18 h following incubation at 37 °C in foetal calf serum-free medium (data not shown). Vehicle controls (0.1% ethanol and 0.1% DMSO) had no significant ($P<0.05$) inhibitory effect on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells.

3.3. The effect of cannabinoid receptor agonists on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells

The nonselective cannabinoid receptor agonist WIN5521 2-2 (10^{-10}–10^{-5} M) and a selective cannabinoid CB_2 receptor agonist JWH 015 (10^{-10}–10^{-5} M) inhibited phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells (Fig. 2). This inhibition was concentration-related and significant ($P<0.05$) over the concentration range 10^{-6}–10^{-5} M ($IC_{1/2max}$, WIN55212-2 = 8.8×10^{-7} M, 95% C.L. = 2.2×10^{-7}–3.5×10^{-6} M, JWH 015 = 1.8×10^{-6} M, 95% C.L. = 1.2×10^{-6}–2.9×10^{-6} M, $n=5$). The nonselective cannabinoid receptor agonist CP55,940 (10^{-10}–10^{-6} M) produced a small, nonsignificant ($P>0.05$) inhibition of interleukin-2 release from human peripheral blood

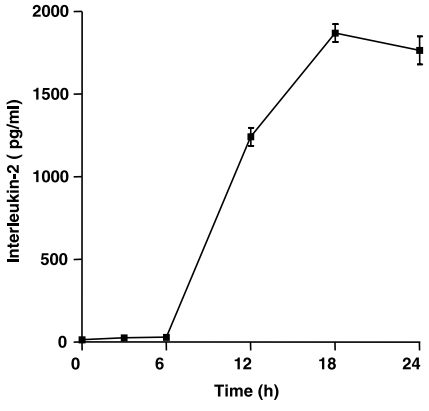

Fig. 1. Time course of phytohaemagglutinin-induced interleukin-2 release from human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were stimulated with phytohaemagglutinin (10 μg/ml) for 3, 6, 12, 18 and 24 h. Cell-free supernatants were harvested for interleukin-2 assay by ELISA as described in Materials and methods. Data are means and S.E.M. of five separate experiments.

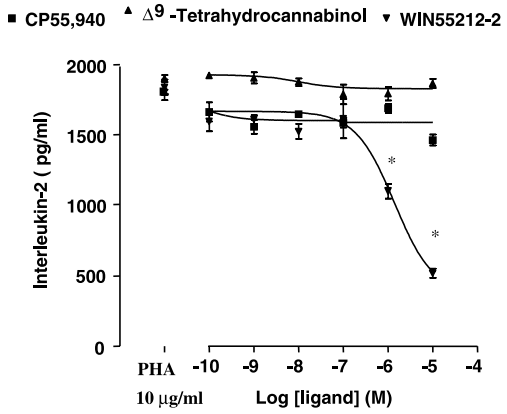

Fig. 2. Effect of nonselective cannabinoid agonists on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were treated with CP55,940 (10^{-10}–10^{-5} M), Δ^9-tetrahydrocannabinol (10^{-10}–10^{-5} M) or WIN55212-2 (10^{-10}–10^{-5} M) for 2 h before stimulation with phytohaemagglutinin (10 μg/ml) for a further 18 h. Cell-free supernatants were harvested and assayed for interleukin-2 by ELISA as described in Materials and methods. Data are means and S.E.M. of five separate experiments. *Denotes significant difference ($P<0.05$) from the control (phytohaemagglutinin-treated cells) (Student's *t*-test).

mononuclear cells (Fig. 2). The nonselective cannabinoid receptor agonist Δ^9-tetrahydrocannabinol (10^{-10}–10^{-6} M) and the selective cannabinoid CB_1 receptor agonist ACEA (10^{-10}–10^{-6} M) also had no significant ($P>0.05$) inhibitory effect on the release of interleukin-2 from human peripheral blood mononuclear cells. As a positive control, dexamethasone (10^{-10}–10^{-6} M), a glucocorticoid, significantly ($P<0.05$) inhibited phytohaemagglutinin-induced interleukin-2 release from human peripheral blood mononuclear cells ($IC_{1/2max} = 1.3 \times 10^{-8}$ M, C.L. $= 5.4 \times 10^{-9}$–3.2×10^{-8} M, $n=5$, Fig. 3). The maximum inhibition produced by JWH 015 was greater than that produced by WIN55212-2 (Fig. 2).

3.4. The effect of SR141716A and SR144528 on WIN55212-2- and JWH 015-induced inhibition of interleukin-2 from human peripheral blood mononuclear cells

When incubated with human peripheral blood mononuclear cells for 18 h, neither SR141716A (10^{-6} M) nor SR144528 (10^{-6} M) had any significant effect on phytohaemagglutinin-induced interleukin-2 release (interleukin-2 release = 1530.5 ± 80.8 pg/ml ($n=5$) and 1653.4 ± 65.5 pg/ml ($n=5$), respectively) when compared with phytohaemagglutinin-treated controls (1655.7 ± 52.8 pg/ml ($n=9$)). SR141716A (10^{-6} M) had no significant ($P>0.05$) effect in attenuating the inhibitory action of WIN55212-2 on phytohaemagglutinin-induced release of interleukin-2 (Fig. 4). In contrast, SR144528 (10^{-6} M) significantly ($P<0.05$, two-way ANOVA followed by Bonferroni's post hoc test, $n=5$) antagonised the inhibitory effects of WIN55212-2 on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells ($pA_2 = 6.3 \pm 0.1$, $n=5$) (Fig. 4).

Similarly, SR141716A (10^{-6} M) had no significant ($P>0.05$)effect in attenuating the inhibitory effect of JWH 015 on phytohaemagglutinin-induced release of interleukin-2. In contrast, SR144528 (10^{-6} M) significantly ($P<0.05$, two-way ANOVA followed by Bonferroni's post hoc test, $n=5$) antagonised the inhibitory effects of JWH 015 on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells ($pA_2 = 6.5 \pm 0.1$, $n=5$) (data not shown).

3.5. The effect of CP55,940 and Δ^9-tetrahydrocannabinol on WIN55212-2-induced inhibition of interleukin-2 from human peripheral blood mononuclear cells

CP55,940 (10^{-6} M) and Δ^9-tetrahydrocannabinol (10^{-6} M) significantly ($P<0.05$, two-way ANOVA followed by Bonferroni's post hoc test, $n=5$) antagonised the inhibitory effects of WIN55212-2 on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells (Figs. 5 and 6). When pA_2 values were calculated from these data, a value of 6.1 ± 0.1 ($n=5$) was obtained for CP55940 and a value of 6.96 ± 0.16 ($n=5$) for Δ^9-tetrahydrocannabinol.

3.6. Effect of CP55,940 on dexamethasone-induced inhibition of interleukin-2 from human peripheral blood mononuclear cells

CP55,940 (10^{-6} M) had no significant ($P>0.05$) effect in antagonising the inhibitory actions of dexame-

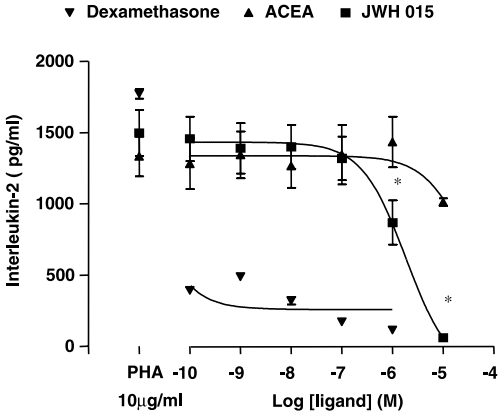

Fig. 3. Effect of selective cannabinoid agonists and dexamethasone on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were treated with ACEA (10^{-10}–10^{-5} M), JWH 015 (10^{-10}–10^{-5} M) or dexamethasone (10^{-10}–10^{-5} M) for 2 h before stimulation with phytohaemagglutinin (10 μg/ml) for a further 18 h. Cell-free supernatants were harvested and assayed for interleukin-2 by ELISA as described in Materials and methods. Data are means and S.E.M. of five separate experiments. *Denotes significant difference ($P<0.05$) from the control (phytohaemagglutinin-treated cells) (Student's *t*-test).

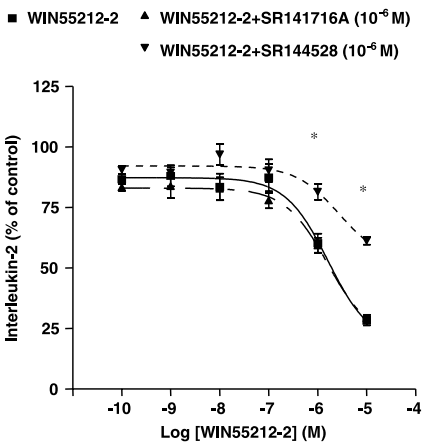

Fig. 4. Effect of SR141716A or SR144528 on WIN55212-2-induced inhibition of interleukin-2 release from human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were preincubated with SR141716A (10^{-6} M) or SR144528 (10^{-6} M) for 30 min before addition of WIN55212-2 (10^{-10}–10^{-5} M) for 2 h. Human peripheral blood mononuclear cells were stimulated with phytohaemagglutinin (10 μg/ml) for further 18 h. Cell-free supernatants were harvested for interleukin-2 assay by ELISA as described in Materials and methods. Data are means and S.E.M. of five separate experiments. *Denotes significant difference from WIN55212-2-treated cells ($P<0.05$, two-way ANOVA followed by Bonferroni's post hoc test, $n=5$).

thasone on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells (Fig. 7).

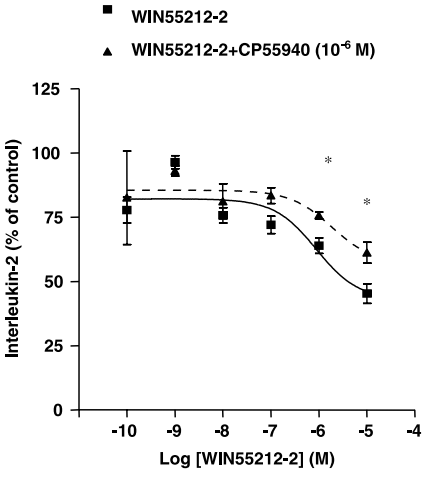

Fig. 5. Effect of CP55940 on WIN55212-2-induced inhibition of interleukin-2 release from human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were preincubated with CP55,940 (10^{-6} M) for 30 min before addition of WIN55212-2 (10^{-10}–10^{-5} M) for 2 h. Human peripheral blood mononuclear cells were stimulated with phytohaemagglutinin (10 μg/ml) for a further 18 h. Cell-free supernatants were harvested for interleukin-2 assay by ELISA as described in Materials and methods. Data are means and S.E.M. of five separate experiments. *Denotes significant difference from WIN55212-2-treated cells ($P<0.05$, two-way ANOVA followed by Bonferroni's post hoc test, $n=5$).

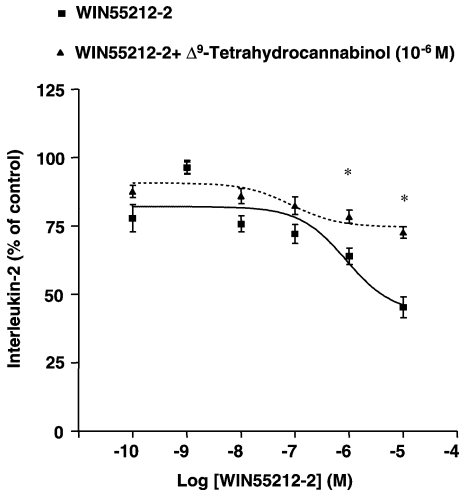

Fig. 6. Effect of Δ^9-tetrahydrocannabinol on WIN55212-2-induced inhibition of interleukin-2 release from human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were preincubated with Δ^9-tetrahydrocannabinol (10^{-6} M) for 30 min before addition of WIN55212-2 (10^{-10}–10^{-5} M) for 2 h. Human peripheral blood mononuclear cells were stimulated with phytohaemagglutinin (10 μg/ml) for a further 18 h. Cell-free supernatants were harvested for interleukin-2 assay by ELISA as described in Materials and methods. Data are means and S.E.M. of five separate experiments. *Denotes significant difference from WIN55212-2-treated cell ($P<0.05$, two-way ANOVA followed by Bonferroni's post hoc test).

3.7. Effect of CP55,940 on the release of interleukin-2 from nonstimulated human peripheral blood mononuclear cells

Addition of CP55,940 (10^{-5} M) to nonstimulated human peripheral blood mononuclear cells followed by incubation at 37 °C for 18 h evoked a minimal release of interleukin-2

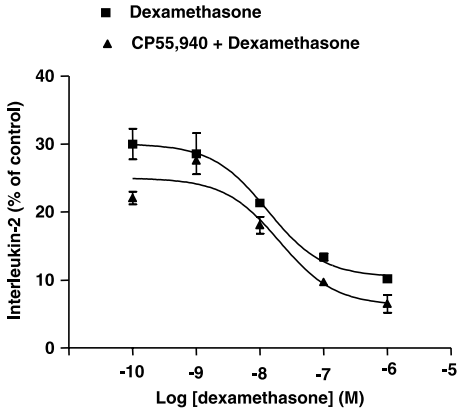

Fig. 7. Effect of CP55,940 on dexamethasone-induced inhibition of interleukin-2 release from human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were preincubated with CP55,940 (10^{-6} M) for 30 min before addition of dexamethasone (10^{-10}–10^{-6} M) for 2 h. Human peripheral blood mononuclear cells were stimulated with phytohaemagglutinin (10 μg/ml) for a further 18 h. Cell-free supernatants were harvested for interleukin-2 assay by ELISA as described in Materials and methods. Data are means and S.E.M. of five separate experiments.

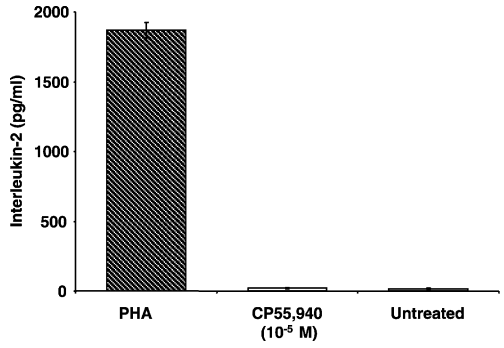

Fig. 8. Effect of CP55,940 on the secretion of interleukin-2 from human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were stimulated with phytohaemagglutinin (10 μg/ml) or CP55,940 (10^{-5} M) for 18 h. Cell-free supernatants were harvested for interleukin-2 assay by ELISA as described in Materials and methods. Data are means and S.E.M. of five separate experiments.

(21.8 ± 6.3 pg/ml, $n = 5$), which was not significantly ($P > 0.05$) different from the basal release (Fig. 8).

4. Discussion

Cannabinoid receptor ligands have potential utility as anti-inflammatory drugs for the treatment of many disease conditions primarily because of their immunosuppressive actions, but their psychoactive effects limit their therapeutic benefits. Emerging evidence suggests that cannabinoids produce many of their immunosuppressive effects by inhibiting T cell responses (for reviews, see Klein et al., 1998a,b; Parolaro, 1999). A significant proportion of these studies have been conducted on cell lines and transfected cells derived from rats or mice (Kaminski et al., 1992; Condie et al., 1996; Massi et al., 2000). While these systems provide useful information for the understanding of the functional properties of cannabinoid receptors, extrapolating these data to man may be hindered by problems of species differences and the artificial nature of the cell lines and transfected cells in which receptors are overexpressed (Kenakin et al., 1995). Consequently, we have investigated the effects of a range of cannabinoid receptor ligands on phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells, a human immune cell.

In the present study, we have shown that a nonselective cannabinoid receptor agonist WIN55212-2 (Felder et al., 1995) and a selective cannabinoid CB_2 receptor agonist JWH 015 (Huffman et al., 1996) evoked a significant concentration-related inhibition of phytohaemagglutinin-induced interleukin-2 release from human peripheral blood mononuclear cells. The nonselective and synthetic cannabinoid CP55,940 (Felder et al., 1995), produced a small, nonsignificant inhibition of interleukin-2 release from human peripheral blood mononuclear cells whereas the plant cannabinoid, Δ^9-tetrahydrocannabinol and the selective cannabinoid CB_1 receptor agonist, ACEA (Hillard et al., 1999), were ineffective in inhibiting phytohaemagglutinin-induced release of interleukin-2. The inhibition of phytohaemagglutinin-induced release of interleukin-2 evoked by WIN55212-2 was not antagonised by pretreatment of the cells with SR141716A, a cannabinoid CB_1 receptor antagonist (Rinaldi-Carmona et al., 1994). However, SR144528, a cannabinoid CB_2 receptor antagonist (Rinaldi-Carmona et al., 1998), significantly attenuated the inhibitory effects of WIN55212-2. Taken together, these data suggest that the observed effects were mediated by a cannabinoid CB_2-like receptor.

Peripheral blood mononuclear cells, used in the present study, comprised 95% lymphocytes and 5% monocytes. In adult blood, lymphocytes comprise approximately 83% of the mononuclear cells (Dien and Lentner, 1970), suggesting that the buffy coat cells used by us contained fewer monocytes than expected, or that the isolation process results in a selective loss of monocytes. The buffy coat cells used by us are a by-product of the preparation of plasma for human use, and it is possible that the more adherent monocytes are lost in the handling of blood to produce plasma and then in the preparation of mononuclear cells by us resulting in a preparation enriched with nonadherent lymphocytes.

In the present study, we cultured human peripheral blood mononuclear cells in foetal calf serum-free medium. While it is conventional to include foetal calf serum in cell culture medium (for example, Corrigan et al., 1995), we chose not to include it because plasma proteins have been shown to bind cannabinoids and reduce their potency (Dewey, 1986), that is, this process acts as an agonist uptake/removal process. Furthermore, if this binding were saturable, over the concentration range studied, then this could influence the data obtained particularly when attempting to characterise antagonist activity (Kenakin and Beek, 1981). Thus, we elected to negate the influence of protein binding in our experiments by omitting foetal calf serum from the medium. When unstimulated peripheral blood mononuclear cells were incubated for 18 h in serum-free medium, no significant change in cell numbers nor a change in cell viability was observed. This may be unexpected since serum contains the growth factors necessary for cell survival and proliferation. However, in our experiments, unstimulated lymphocytes released a small, nonsignificant amount of interleukin-2. This basal release of interleukin-2 may have been sufficient to maintain lymphocytes in a viable, functional state but be insufficient to promote cell replication.

In the present study, inhibition of phytohaemagglutinin-induced release of interleukin-2 by WIN55212-2 and JWH 015 was observed at concentrations greater than those required to displace a radiolabelled cannabinoid receptor ligand in receptor binding studies (>1 μM) (Felder et al., 1995; Showalter et al., 1996). However, the potency of WIN55212-2 in the present study is similar to that reported by others in studies on a murine macrophage cell line (RAW264.7) (Ross et al., 2000). It is noteworthy that the

K_d values reported from cannabinoid binding studies are usually higher in experiments where purified receptors or transfected cells have been used (Howlett, 1995; Slipetz et al., 1995). This difference has been ascribed to loss of activity of lipophilic cannabinoids due to nonspecific interactions with cells and serum (Howlett, 1995; Slipetz et al., 1995). Furthermore, the pA_2 value for the cannabinoid CB_2 receptor antagonist SR144528 reported in this study is significantly lower than the pK_i value reported for this compound on Chinese hamster ovary cells transfected with CB_2 receptors (Iwamura et al., 2001). It is lower than that previously obtained by us in studies on epithelial cells (Ihenetu et al., 2003), although the potency of SR144528 in the present study is similar to that reported by others in experiments on a murine macrophage cell line (Ross et al., 2000). One explanation for this difference may be due to the level of cannabinoid CB_2 receptor expression in mononuclear cells compared to that in other tissues, coupled with the lipophilic nature of these compounds reducing the actual concentration of antagonist available at the receptor. Clearly, further experiments are required to determine why SR144528 is apparently less potent as a cannabinoid CB_2 receptor antagonist on monocytes compared with other tissues.

In line with the present study, it is noteworthy that few studies to date have reported functional effects of cannabinoids via cannabinoid CB_2 receptors at concentrations less than 1 μM (Ross et al., 2000). Furthermore, in transfected cell lines, the stoichiometry of key regulatory proteins may be altered resulting in responses distinct from those found in primary cells (Kenakin et al., 1995). Thus, it seems possible that our finding that cannabinoid agonists were less potent in human peripheral blood mononuclear cells when compared to data published by others may reflect a low level of cannabinoid receptor expression in these cells.

Other published work suggests that cannabinoids can stimulate cytokine release. In contrast to our findings, Derocq et al. (1995) were able to show that low concentrations of CP55,940 significantly ($P < 0.05$) increased DNA synthesis in human tonsilar B cells, a primary cell system that expresses high levels of cannabinoid CB_2 receptors (Galiegue et al., 1995). Other studies showing effects of cannabinoids at low concentrations include experiments in which the cannabinoid receptor agonists CP55,940 or WIN55212-2 caused increased expression of IL-8 in HL-60 cells transfected with cannabinoid CB_2 receptors (Jbilo et al., 1999; Derocq et al., 2000). However, these cannabinoid CB_2 receptor agonists still increased IL-8 expression when wild type HL-60 cells were used (Derocq et al., 2000; Jbilo et al., 1999). These findings suggest that HL-60 cells have a higher level of endogenous cannabinoid CB_2 receptor expression than human peripheral blood mononuclear cell since, in the present study, the cannabinoid receptor agonist CP55,940 did not induce the release of interleukin-2 from peripheral blood mononuclear cells, even after incubation for 18 h.

Other published work has also shown that cannabinoids may either increase or decrease interleukin-2 release from immune cells depending on the experimental conditions and the cells studied (Pross et al., 1992; Watzl et al., 1991). In the murine lymphocyte cell line, EL4.IL-2, Δ^9-tetrahydrocannabinol and cannabidiol inhibited phorbol myristyl acetate/ionophore-induced interleukin-2 mRNA expression and interleukin-2 release in a concentration-dependent manner (Condie et al., 1996; Jan et al., 2002). In contrast, in phytohaemagglutinin-activated human peripheral blood mononuclear cells, Δ^9-tetrahydrocannabinol and cannabidiol did not inhibit interleukin-2 release, although these cannabinoid receptor ligands did inhibit the release of other cytokines (Watzl et al., 1991), findings that are consistent with those reported in the present study. Thus, it appears that the choice of cell and the stimulus used to provoke cytokine release may influence the inhibitory activity of cannabinoid receptor agonists. Such an effect is not unique to cannabinoid receptor agonists and has been noted in studies with other classes of agonists (e.g. Kenakin, 1982; Kenakin et al., 1995). The exact reason for the differences between the findings of the present study and those described above is still unclear and additional experiments are necessary to resolve these discrepancies.

We and others have shown that a range of cannabinoid ligands including WIN55212-2, CP55,940 and Δ^9-tetrahydrocannabinol act as agonists at the peripheral cannabinoid CB_2 receptor to cause inhibition of tumour necrosis factor-α-induced release of interleukin-8 in HT-29 cells (Ihenetu et al., 2001) and to inhibit adenylate cyclase activity in Chinese hamster ovary cells transfected with cannabinoid CB_2 receptors (Bayewitch et al., 1995), respectively. However, in the present study, CP55,940 only marginally and nonsignificantly inhibited phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells while Δ^9-tetrahydrocannabinol had no effect in inhibiting this release. Receptor binding studies have demonstrated that these two agonists have affinity for cannabinoid CB_2 receptors on immune cells (Bouaboula et al., 1993; Galiegue et al., 1995; Kaminski et al., 1992). Thus, one explanation for this lack of activity could be due to a low level of efficacy combined with a relatively low level of cannabinoid CB_2 receptor expression. Similar effects have been reported in experiments with partial agonists in other receptor systems (Kenakin and Beek, 1982). This hypothesis is supported by the ability of CP55,940 and Δ^9-tetrahydrocannabinol to inhibit the effects of WIN55,212-2. In the present study, both compounds shifted concentration–effect curves for WIN55,212-2-induced inhibition of interleukin-2 release, to the right. In the case of CP55,940, the small inhibitory effect on interleukin-2 release adds further weight to the hypothesis that it is acting as a weak partial agonist at cannabinoid CB_2 receptors relative to the effect observed with WIN55212-2.

Given the apparent potency of CP55,940 at cannabinoid CB_2 receptors, reported by others (Showalter et al., 1996), it

is possible that the lack of inhibitory effect on phytohaemagglutinin-induced interleukin-2 release is because the inhibitory effect is negated by additional release of interleukin-2 induced by CP55,940. Such an effect has been reported by others (Jbilo et al.,1999) and could also explain the apparent antagonism of the inhibitory action of WIN55212-2 by CP55,940. However, this is clearly not the case since when human peripheral blood mononuclear cells were incubated with CP55,940 for 18 h, no release of interleukin-2 was seen, adding support to the hypothesis that, in our experiments, CP55,940 acts at cannabinoid CB_2 receptors on human peripheral blood mononuclear cells to antagonise the effects of WIN55212-2.

To test the specificity of CP55,940 in antagonising the effect of WIN55,212-2, we studied the effect of CP55,940 in antagonising dexamethasone-evoked inhibition of phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells. CP55,940 did not antagonise dexamethasone-evoked inhibition of phytohaemagglutinin-induced release of interleukin-2 but marginally potentiated its effect. In order to investigate whether high concentration of CP55,940 evoked the release of interleukin-2 on its own, a point which could account for its poor activity in inhibiting phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells, we studied the effect of CP55,940 (10^{-5} M) on the release of interleukin-2 from human peripheral blood mononuclear cells in the absence of phytohaemagglutinin. In these experiments, CP55,940 alone did not stimulate the release of interleukin-2 from phytohaemagglutinin. Taken together, these results show that CP55,940 appears to be specific in antagonising WIN55212-2-mediated inhibition of phytohaemagglutinin-induced interleukin-2 release from human peripheral blood mononuclear cells and does not, on its own, evoke the release of interleukin-2. Δ^9-Tetrahydrocannabinol exhibited similar profiles (data not shown). Previously, other laboratories have demonstrated that Δ^9-tetrahydrocannabinol antagonised HU293a and HU210 (nonselective cannabinoid receptor agonists) induced inhibition of forskolin-stimulated adenylyl cyclase in Chinese hamster ovary cells transfected with CB_2 receptors (Bayewitch et al., 1996). To our knowledge, the present study is the first report of CP55,940 acting as a partial agonist/antagonist at a cannabinoid CB_2 receptor-mediated event in a native system.

In summary, we have demonstrated that WIN55212-2 and JWH 015 evoke inhibition of interleukin-2 release from human peripheral blood mononuclear cells. The selective cannabinoid CB_2 receptor antagonist SR144528 antagonised WIN55212-2 inhibition of phytohaemagglutinin-induced release of interleukin-2 from human peripheral blood mononuclear cells whereas the cannabinoid CB_1 receptor antagonist SR141716A had no effect. Furthermore, CP55,940 and Δ^9-tetrahydrocannabinol behaved as partial agonists/antagonists under our experimental conditions, indicating that they possess affinity for, but low efficacy at, cannabinoid CB_2 receptors. Thus, this study adds to and extends the body of knowledge suggesting that cannabinoids modulate immune cell function and suggests that some ligands have partial agonist activity at cannabinoid CB_2 receptors. The structures of the cannabinoid receptor ligands utilised in the above study could therefore serve as models for the synthesis of novel and more selective cannabinoid compounds for therapeutic use.

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