J Neuroimmune Pharmacol (2007) 2:178–183
DOI 10.1007/s11481-006-9040-4

BRIEF REPORT

WIN55,212-2-Mediated Inhibition of HIV-1 Expression in Microglial Cells: Involvement of Cannabinoid Receptors

R. Bryan Rock · Genya Gekker · Shuxian Hu · Wen S. Sheng · Guy A. Cabral · Billy R. Martin · Phillip K. Peterson

Received: 14 April 2006 / Accepted: 15 August 2006 / Published online: 12 October 2006

Abstract Cannabinoid receptors CB_1 and CB_2 are primarily expressed in cells of the nervous and immune systems, respectively. Recently, the synthetic CB_1/CB_2 agonist WIN55,212-2 was found to suppress replication of HIV-1 in microglial cell cultures. The present study was undertaken to test the hypothesis that WIN 55,212-2's antiviral effect is mediated *via* CB_2 receptors. By reverse transcription-polymerase chain reaction, microglia were found to express both CB_1 and CB_2 receptors. Using additional CB_1/CB_2 receptor agonists and selective antagonists, we found that CB_2 receptors are involved in WIN55,212-2's antiviral activity and surprisingly that the CB_1 receptor-selective antagonist SR141716A behaved as an agonist in these brain macrophages.

Human fetal brain tissue was obtained under the protocol approved by the Human Subjects Research Committee at our institution.

R. B. Rock · G. Gekker · S. Hu · W. S. Sheng · P. K. Peterson
Center for Infectious Diseases and Microbiology Translational Research and Department of Medicine,
University of Minnesota Medical School,
Minneapolis, MN, USA

G. A. Cabral
Department of Microbiology and Immunology,
Virginia Commonwealth University,
Richmond, VA, USA

B. R. Martin
Department of Pharmacology and Toxicology,
Virginia Commonwealth University,
Richmond, VA, USA

P. K. Peterson (✉)
Division of Infectious Diseases and International Medicine,
Department of Medicine, University of Minnesota,
MMC250, 420 Delaware Street S.E.,
Minneapolis, MN 55455, USA
e-mail: peter137@umn.edu

Key words cannabinoids · HIV-1 · microglia · WIN55,212-2 · SR141716A

Introduction

Two types of G-protein coupled cannabinoid receptors, CB_1 and CB_2, have been well characterized (Pertwee 2006), and although the healthy nervous system is richly endowed with CB_1 receptors and appears to be relatively devoid of CB_2 receptors, CB_2 receptors predominate in the immune system (Cabral and Dove Pettit 1998; Klein et al. 2003). The immunosuppressive effects on T lymphocytes and macrophages by Δ^9-tetrahydrocannabinol, the main psychotropic constituent of cannabis (marijuana), appear to be largely related to its activity at CB_2 receptors (Cabral and Dove Pettit 1998; Klein et al. 2003). The consequences of these immunological impairments have been shown in animal models to promote the pathogenesis of infections caused by intracellular microorganisms (Cabral and Dove Pettit 1998; Klein et al. 2003).

Our laboratory has been interested in the effects of substances of abuse on the expression of HIV-1 in microglia, which are the major cell type productively infected by this virus in the central nervous system. In a recent study, we found that in contrast to morphine, which up-regulated HIV-1 expression, the synthetic nonselective CB_1/CB_2 receptor agonist WIN55,212-2 inhibited viral replication (Peterson et al. 2004).

The purpose of the present study was to characterize the cannabinoid receptor types present in human fetal brain-derived microglia and to determine which cannabinoid receptor type is involved in the anti-HIV-1 activity of WIN55,212-2. Based on literature indicating the primacy of CB_2 receptors in cannabinoid-mediated immunomodulation

and data that WIN55,212-2 has some degree of selectivity for CB_2 receptors (Pertwee 1997), we hypothesized that CB_2 receptors would be the main target of WIN55,212-2. Although support for this hypothesis was found, the results of this study also demonstrated that the CB_1-selective antagonists SR141716A and AM-251 behave as agonists in microglia.

Materials and methods

Reagents The following reagents were purchased from the source as indicated: Dulbecco's modified Eagle's medium (DMEM), Hanks' balanced salt solutions (HBSS), trypsin, penicillin, streptomycin, and WIN55,212-2 [(*R*)-(+)-[2, 3-dihydro-5-methyl-3-(4-morpholinylmethyl)] pyrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate] (Sigma, St. Louis, MO, USA); fetal bovine serum (FBS) (Hyclone, Logan, UT, USA); CP55,940 [(−)-*cis*-3*R*-[2-Hydroxy-4-(1,1-demethylheptyl)phenyl]-*trans* 4*R*-3(3-hydroxypropyl)-1*R*-cyclohexanol], were kindly provided by Raj K. Razdan (Organix, Inc.); WIN55,212-3 [*S*(−)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone mesylate], SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorphenyl)-4-methyl-1*H*-pyrozole-3-carboxamidehydrochloride], and SR144528 [*N*-(1*S*) -endo-1,3,3-trimethylbicyclo [2.2.1]heptan-2-yl] 5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide)] were kindly provided by S. Thayer, University of Minnesota; AM-251 [*N*-(piperdin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide] (Tocris Bioscience, Ellisville, MO, USA); JWH 0-15 [(2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone] was kindly provided by Billy Martin, Virginia Commonwealth University.

Microglial cell cultures Highly enriched cultures of human microglia were isolated from fetal brain tissue specimens by using a protocol approved by the Human Subject Research Committee at our institution as previously described (Peterson et al. 2004). Briefly, brain tissues were dissociated after trypsinization (0.25%) and plated into 75-cm^2 Falcon culture flasks in DMEM containing 10% FBS, penicillin (10 U/ml), and streptomycin (100 U/ml). The medium was replenished 4 days later. Microglia were harvested after 10–14 days with purity of > 99% (stained by anti-CD68 antibody).

Drug treatment and viral infection Microglial cultures (1×10^5 cells/well in a 48-well plate) were pretreated without (control) or with CB_1/CB_2 receptor agonists (WIN55,212-2 and CP55,940), CB_2-selective agonist JWH 0-15, CB_1-selective antagonists SR141716A and AM-251, CB_2-selective antagonist SR144528, or the inactive enantiomer WIN55,212-3 (Savinainen et al. 2005) for 3 h prior to HIV-1 infection. In one experiment SR144528 (a CB_2-selective antagonist) (Howlett et al. 2002) was added 30 min prior to treatment with CP55,940 or WIN55,212-2. All CB receptor ligands were constituted in DMSO with the exception of CP55,940, which was constituted in ethanol. Untreated (control) cultures contained equivalent concentrations of these diluents.

HIV-1_{SF162}, a monocytotropic (R5) variant, was obtained from the NIH AIDS Research and Reference Reagent Program (National Institute of Allergy and Infectious Diseases, Bethesda, MD) and was added to microglia at a multiplicity of infection (MOI) of 0.02. After 24 h of absorption with HIV-1 at 37°C, cannabinoid-treated and untreated control cells were washed three times and resuspended in culture medium (DMEM, 10% FBS) containing the indicated concentrations of drugs or drug combinations.

Assessment of HIV-1 expression Supernatants from microglial cell cultures were collected at 7 days postinfection and assayed for HIV-1 p24 Ag production by enzyme-linked immunosorbent assay (Beckman Coulter, Fullerton, CA, USA) as previously described (Peterson et al. 2004).

Cell viability To assess the effect of cannabinoid receptor ligands on cell viability, two assays were used: microscopic evaluation of trypan blue dye exclusion and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide mitochondrial dehydrogenase) (Sigma) assays. MTT was added to cell cultures at a final concentration of 1 mg/ml, and after 4 h of incubation, the assay was stopped by adding lysis buffer [20% SDS (w/v) in 50% *N*, *N*-dimethyl formamide, pH 4.7] followed by overnight incubation. The absorbance (OD) measured at 600 nm reflects mitochondrial integrity.

RT-PCR assay for cannabinoid receptor expression Total RNA isolated with Qiagen RNeasy Mini kit (Qiagen, Valencia, CA) from microglia was treated with DNase (Ambion, Austin, TX) followed by reverse transcription (RT) for cDNA synthesis. Total RNA was reverse-transcribed to cDNA with SuperScript™ II reverse transcriptase, 5× cDNA synthesis buffer, 0.1 M dithiothreitol (DTT) (Invitrogen, Carlsbad, CA, USA), dNTP, oligo $(dT)_{12–15}$ and random primer (Amersham Biosciences, Piscataway, NJ, USA) to a final volume of 20 μl. The same amounts of RNA were used in no RT reaction as controls to monitor DNA contamination. The cDNA was amplified by RT-polymerase chain reaction (RT-PCR) (FastStart DNA Master Plus SYBR Green detection with Roche LightCycler 2.0; Roche, Indianapolis, IN, USA, USA) with primers specific for CB_1 and CB_2 receptors. The

amplified PCR products were electrophoresed for size verification. The primer sequences were: 5′-GCGTACTGCTTCTGTT CATCGTGTAT-3′ (sense) and 5′-GCAGGTCCTTACTC CTCAGAGCATAG-3′ (antisense) for CB_1; 5′-CCTCTCA GCACTAGTCTCCTACCTGC-3′ (sense) and 5′-TCCACT CCGTAGAGCATAGATGACAG-3′ (antisense) for CB_2. The amplified PCR products were 363 and 436 bp, respectively. The amplification protocols were 95°C: 10 s, 67°C: 10 s and 72°C: 18 s for CB_1 and CB_2 receptors.

Assessment of CCR5 The effects of cannabinoid ligands on microglial cell expression of the chemokine receptor CCR5 was examined by flow cytometry. Microglia (3×10^5/0.5 ml medium) were incubated for 48 h in Teflon-coated vials in the absence (control) or presence of WIN55,212-2 or WIN55,212-3. Cells were then stained with fluorescein isothiocyanate-labeled anti-CCR5 antibody followed by flow cytometric analysis. Data are expressed as the percentage of microglia positive for CCR5 by using the FACSDiva software provided by the manufacturer (BD Biosciences, San Jose, CA, USA).

Statistical analysis For analysis of the effect of cannabinoid receptor ligands on viral expression, p24 Ag levels in supernatants of cells treated with cannabinoid agonists and antagonists were expressed as % inhibition relative to p24 Ag levels in supernatants of untreated (control) cells. A mixed-effects, repeated-measures model was used that accounts for within-person correlations and intrinsic differences among individuals. The Tukey method was applied to adjust for multiple comparisons, and the Mixed Procedure in SAS version 8.2 was used to perform this analysis. Analysis of the effect of WIN55,212-2 on CCR5 expression involved the Student *t*-test as a measure of percent inhibition of CCR5 relative to control (untreated) microglia.

Results

Effect of CP55,940 on HIV-1 expression in microglia Of the cannabinoid agonists that we had studied previously, only WIN55,212-2 potently inhibited HIV-1 expression (Peterson et al. 2004). As WIN55,212-2 binds with somewhat higher affinity to CB_2 than CB_1 receptors, we evaluated the nonselective CB_1/CB_2 receptor agonist CP55,940, a synthetic cannabinoid that binds with high affinity to both receptors. Suppression of viral expression by CP55,940 was concentration-dependent (Fig. 1) and similar to that previously observed with WIN55,212-2 (Peterson et al. 2004). Maximal inhibition (approximately 40% at 10^{-6} M), was comparable to WIN55,212-2 (Fig. 1).

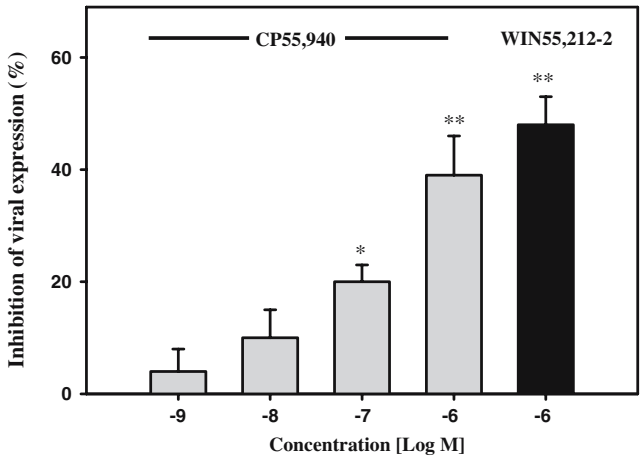

Fig. 1 Concentration response of CP55,940 on HIV-1 expression. Microglia were incubated in culture medium alone (control) or were treated with indicated concentrations of CP55,940 or WIN55,212-2 for a total of 7 days. Vehicle (DMSO) had no effect on HIV-1 expression compared to medium alone (data not shown). Data are expressed as percent of control (untreated cells) p24 Ag values (6.3 ± 1.3 ng/ml, mean ± SEM; $N = 3$ experiments using microglia from different brain tissue specimens). *$P < 0.05$, **$P < 0.01$ *versus* control.

Microglial cell viability was assessed at 7 days of incubation in medium alone (control), CP55,940 (10^{-6} M), or WIN55,212-2 (10^{-6} M), and >98% of cells were viable in all treatment groups by trypan blue dye exclusion and MTT assays.

Cannabinoid receptors in microglia Microglia isolated from five different brain tissue specimens were evaluated by RT-PCR for CB_1 and CB_2 receptor mRNA. As shown in Fig. 2a, microglia isolated from each of the five brain specimens contained mRNA for both CB_1 and CB_2 receptors.

Effects of CB_1 and CB_2 receptor agonists/antagonists on HIV-1 expression Next, the effects of CB_1 and CB_2 receptor antagonists were compared to WIN55,212-2 and CP55,940. Microglia were treated with CB_1/CB_2 receptor agonists (CP55,940, WIN55,212-2), a CB_2-selective agonist (JWH 0-15), and CB_1-selective (SR141716A) and CB_2-selective (SR144528) receptor antagonists at concentrations ranging between 10^{-9} and 10^{-6} M (Howlett et al. 2002). The inactive, neutral enantiomer of WIN55,212 (WIN55,212-3) was used as a control (Savinainen et al. 2005). The results are shown for the most potent concentration (10^{-6} M) for each of the agonists that had anti-HIV-1 activity (Fig. 2b). WIN55, 212-2 significantly inhibited HIV-1 expression as compared to its inactive enantiomer WIN55,212-3, which suggests that the inhibition of viral expression is receptor-mediated. The CB_2-selective agonist JWH 0-15 had a similar degree of viral expression inhibition as CP55,940. While the CB_2 receptor antagonist SR144528 had no effect

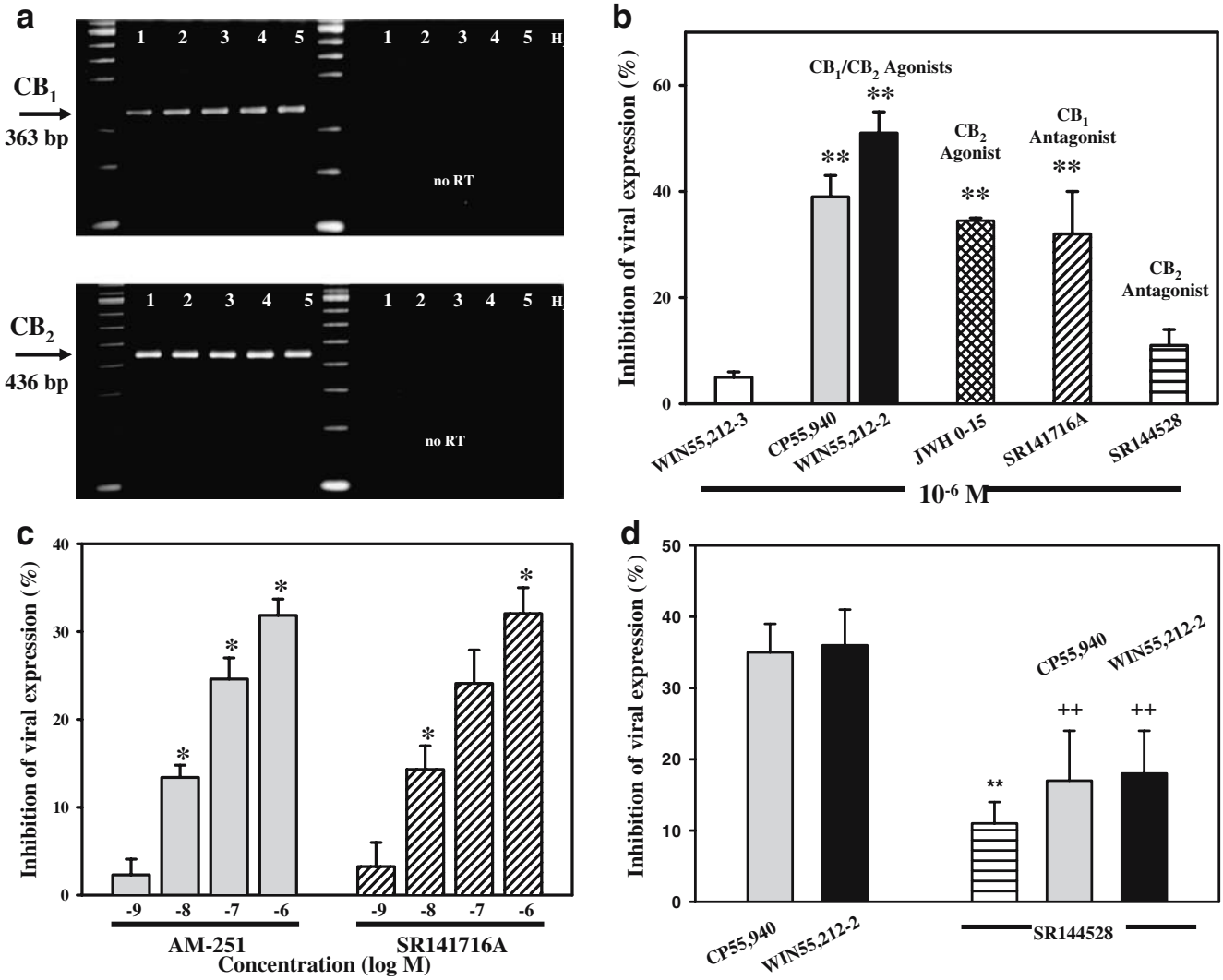

Fig. 2 CB_1 and CB_2 receptors and the effects of cannabinoid receptor ligands on HIV-1 expression in microglia. (a) Total RNA from microglia obtained from five different brain specimens (lanes 1–5) were reverse-transcribed with RT to cDNA followed by RT-PCR amplification. Amplified PCR products were electrophoresed for CB_1 (upper gel) and CB_2 receptors (lower gel). RNA from each specimen was also reverse transcribed without RT as controls to monitor for DNA contamination. (b) Effects of CB_1 and CB_2 receptor agonists and antagonists on HIV-1 expression in microglia. Microglia were incubated in culture medium alone (control) or treated with indicated cannabinoid receptor ligands (10^{-6} M) for a total of 7 days. Data are expressed as percent of control p24 Ag values (6.3 ± 1.3 ng/ml, mean ± SEM; N = 3 experiments using microglial cells from different brain tissue specimens). *P < 0.05, **P < 0.01 *versus* control. (c) Concentration response of AM-251 and SR141716A on HIV-1 expression. Microglia were incubated in culture medium alone (control) or were treated with indicated concentrations of AM-251 or SR141716A for a total of 7 days. Data are expressed as percent of control (untreated cells) p24 Ag values (0.23 ± 0.01 ng/ml, mean ± SEM; N = 2 experiments using microglia from different brain tissue specimens). *P < 0.05, **P < 0.01 *versus* control. (d) Effect of CB_2 receptor antagonist SR144528 on CP55,940 and WIN55,212-2-mediated inhibition of HIV-1 expression in microglia. Microglia were incubated in culture medium alone (control) or were treated with 10^{-6} M CP55,940, WIN55,212-2, or SR144528 alone, or were pretreated with SR144528 prior to CP55,940 or WIN55,212-2 and assessed after 7 days. Data are expressed as percent of control p24 Ag values (6.7 ± 3 ng/ml, mean ± SEM; N = four experiments using microglial cells from different brain tissue specimens). **P < 0.01, *versus* control; ++P < 0.01 versus CP55,940 or WIN 55,212-2 alone.

on HIV-1 expression, surprisingly, the CB_1 receptor antagonist SR141716A inhibited viral expression to a degree similar to that seen with CP55,940. This effect was further characterized by evaluating another CB_1-selective antagonist AM-251, which had a similar inhibitory effect on HIV-1 expression to SR141716A (Fig. 2c). Additionally, the CB_2-selective antagonist SR144528 did not abrogate the inhibitory effect on HIV-1 expression of SR141716A, nor did the CB_1-selective antagonist AM-251 abrogate the inhibitory effect on HIV-1 expression of WIN55,212-2 (data not shown).

Because SR144528 by itself did not inhibit viral expression, microglia were pretreated with SR144528 prior to adding WIN55,212-2 or CP55,940. SR144528 significantly

blocked the antiviral effect of both nonselective agonists (Fig. 2d), supporting the hypothesis that the mechanism of inhibition of viral expression in microglia involves CB_2 receptors.

Effect of the enantiomers of WIN55,212 on expression of CCR5 in microglia Based on previous studies on other psychotropic agents showing modulation of CCR5 in their effects on viral replication in macrophages (Guo et al. 2002; Szabo et al. 2003), we evaluated the effect of WIN55,212-2 on this coreceptor for HIV-1 entry into microglia. Microglia were treated with WIN55,212-2 or its inactive, neutral enantiomer WIN55,212-3 (Savinainen et al. 2005), and the percentage of microglia expressing CCR5 was compared to control cells as determined by flow cytometry (Fig. 3). WIN55,212-2 inhibited CCR5 (39.3 ± 8.3%, mean ± SEM), whereas treatment with the WIN55, 212-3 had little effect on CCR5 expression (5.1 ± 14.4% inhibition). The stereoselectivity of this response is a strong indication of a receptor-mediated process being involved. The interference with viral entry by down-regulation of CCR5 is one potential mechanism by which WIN55,212-2 inhibits HIV-1 expression in microglia.

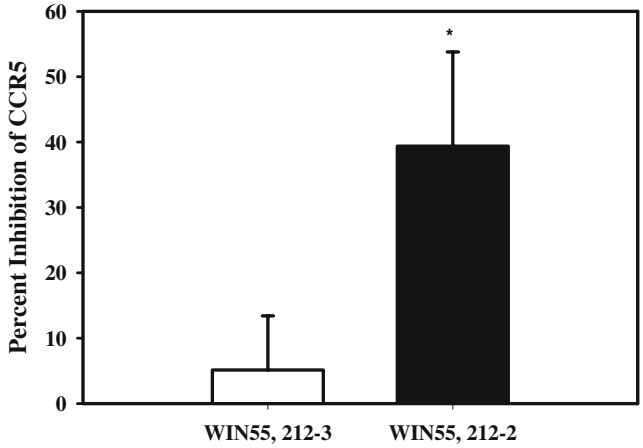

Fig. 3 Effect of the enantiomers of WIN55,212 on expression of CCR5 in microglial. Microglia were incubated for 48 h in the absence or presence of WIN55,212-2 (10^{-6} M) or the inactive enantiomer WIN55,212-3 (10^{-6} M). Cells were then stained with fluorescein isothiocyanate-labeled anti-CCR5 antibody followed by flow cytometry analysis. Data are expressed as percent inhibition of CCR5 relative to control (untreated) microglia (23 ± 8.5%, mean ± SEM; $N = 5$ experiments using microglial cells from different brain tissue specimens). *$P < 0.05$ *versus* control.

Discussion

The purpose of this study was to characterize the cannabinoid receptor types present in human fetal brain-derived microglia and to determine which cannabinoid receptor type is involved in the anti-HIV-1 activity of WIN55,212-2. CB_1 and CB_2 receptors have been identified in macrophages/microglia isolated from rodent brain (Sinha et al. 1998; Carlisle et al. 2002; Facchinetti et al. 2003; Cabral and Marciano-Cabral 2005), whereas CB_2 receptor mRNA was exclusively present in microglia obtained from adult human brain surgical specimens (Klegeris et al. 2003). The finding of CB_1 and CB_2 receptors in fetal brain-derived microglia could thus represent an age-related phenomenon or simply that CB_1 and CB_2 receptors are up-regulated during the preparation of the microglial cell cultures.

Although we hypothesized that WIN55,212-2 was acting through CB_2 receptors, the presence of both CB_1 and CB_2 receptor mRNA expression in microglia raised the question of whether CB_1 or CB_2 was involved. In the course of studying this question, four observations were made: (1) the inhibitory effect of WIN55,212-2 on HIV-1 expression is not unique, in that CP55,940 also suppressed viral expression; (2) CB_2 receptors are involved in WIN55,212-2 inhibitory effect, as witnessed by significant blockade with the CB_2 receptor-selective antagonist SR144528 and the similar inhibitory effect of JWH 0-15, which has been shown to inhibit microglia activation (Ehrhart et al. 2005), suggesting that suppressed microglial activation and decreased HIV-1 replication may be related; (3) down-regulation of CCR5 receptors appeared to be one mechanism whereby WIN55,212-2 inhibits HIV expression, and (4) the involvement of CB_1 in this process cannot be adequately assessed because of the surprising inhibitory effect of the CB_1 receptor-selective antagonists SR141716A and AM-251. This paradoxical agonist activity of SR141716A has been previously identified in other macrophage/microglia models (Gross et al. 2000; Puffenbarger et al. 2000), including a report in which SR141716A's inhibitory effect on multiplication of intracellular *Brucella suis* in human monocytes prompted the authors to suggest that this cannabinoid be considered for treatment of infections caused by intracellular gram-negative bacteria (Gross et al. 2000). The observation in the present study that agents such as WIN55,212-2, CP55,940, and SR141716A (which has also been shown to be effective in the treatment of obesity (Despres et al. 2005) and is about to be marketed under the trade name Rimonabant®), have the capacity to inhibit HIV-1 expression should foster further exploration of cannabinoids as potential antiviral agents. On the other hand, results from animal models raise concern regarding possible effects of the natural cannabinoid Δ-THC in promoting HIV-1 infection (Baldwin and Roth 2005).

Acknowledgements This work was supported by grants from National Institute on Drug Abuse, DA-04381 and DA-09789, and was supported in part by U.S. Public Health Service grant DA-04381.

References

Baldwin GC, Roth MD (2005) Biological links between cannabinoids and HIV infection. In: Friedman H (ed) Infectious Diseases and Substance Abuse. Kluwer Academic/Plenum Publishers, New York, pp 13–32

Cabral GA, Dove Pettit DA (1998) Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. J Neuroimmunol 83:116–123

Cabral GA, Marciano-Cabral F (2005) Cannabinoid receptors in microglia of the central nervous system: immune functional relevance. J Leukoc Biol 78:1192–1197

Carlisle SJ, Marciano-Cabral F, Staab A, Ludwick C, Cabral GA (2002) Differential expression of the CB_2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. Int Immunopharmacol 2:69–82

Despres JP, Golay A, Sjostrom L (2005) Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. N Engl J Med 353:2121–2134

Ehrhart J, Obregon D, Mori T, Hou H, Sun N, Bai Y, Klein T, Fernandez F, Tan J, Shytle RD (2005) Stimulation of cannabinoid receptor 2 (CB_2) suppresses microglial activation. J Neuroinflammation 2:29

Facchinetti F, Del Giudice E, Furegato S, Passarotto M, Leon A (2003) Cannabinoids ablate release of TNF-alpha in rat microglial cells stimulated with lypopolysaccharide. Glia 41:161–168

Gross A, Terraza A, Marchant J, Bouaboula M, Ouahrani-Bettache S, Liautard JP, Casellas P, Dornand J (2000) A beneficial aspect of a CB_1 cannabinoid receptor antagonist: SR141716A is a potent inhibitor of macrophage infection by the intracellular pathogen *Brucella suis*. J Leukoc Biol 67:335–344

Guo CJ, Li Y, Tian S, Wang X, Douglas SD, Ho WZ (2002) Morphine enhances HIV infection of human blood mononuclear phagocytes through modulation of beta-chemokines and CCR5 receptor. J Investig Med 50:435–442

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54:161–202

Klegeris A, Bissonnette CJ, McGeer PL (2003) Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB_2 receptor. Br J Pharmacol 139:775–786

Klein TW, Newton C, Larsen K, Lu L, Perkins I, Nong L, Friedman H (2003) The cannabinoid system and immune modulation. J Leukoc Biol 74:486–496

Pertwee RG (1997) Pharmacology of cannabinoid CB_1 and CB_2 receptors. Pharmacol Ther 74:129–180

Pertwee RG (2006) Cannabinoid pharmacology: the first 66 years. Br J Pharmacol 147(Suppl 1):S163–S171

Peterson PK, Gekker G, Hu S, Cabral G, Lokensgard JR (2004) Cannabinoids and morphine differentially affect HIV-1 expression in CD4(+) lymphocyte and microglial cell cultures. J Neuroimmunol 147:123–126

Puffenbarger RA, Boothe AC, Cabral GA (2000) Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. Glia 29:58–69

Savinainen JR, Kokkola T, Salo OM, Poso A, Jarvinen T, Laitinen JT (2005) Identification of WIN55212-3 as a competitive neutral antagonist of the human cannabinoid CB_2 receptor. Br J Pharmacol 145:636–645

Sinha D, Bonner TI, Bhat NR, Matsuda LA (1998) Expression of the CB_1 cannabinoid receptor in macrophage-like cells from brain tissue: immunochemical characterization by fusion protein antibodies. J Neuroimmunol 82:13–21

Szabo I, Wetzel MA, Zhang N, Steele AD, Kaminsky DE, Chen C, Liu-Chen LY, Bednar F, Henderson EE, Howard OM, Oppenheim JJ, Rogers TJ (2003) Selective inactivation of CCR5 and decreased infectivity of R5 HIV-1 strains mediated by opioid-induced heterologous desensitization. J Leukoc Biol 74:1074–1082