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Characterization of human cannabinoid CB_2 receptor coupled to chimeric $G\alpha_{qi5}$ and $G\alpha_{qo5}$ proteins

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ABSTRACT

Cannabinoid CB_2 receptors may couple to a variety of G proteins and intracellular effector systems to regulate physiological and pathophysiological processes involved in inflammatory and neuropathic pain. In this study, the coupling of cannabinoid hCB_2 receptors to $G\alpha_{qo5}$ and $G\alpha_{qi5}$ proteins was studied and compared by investigating the pharmacological properties of HEK-293 cells co-expressing cannabinoid hCB_2 with chimeric $G\alpha_{qo5}$ (HEK-hCB_2-G_{qo5}) or $G\alpha_{qi5}$ (HEK-hCB_2-G_{qi5}). Both cell lines were found to be amendable for measuring cannabinoid CB_2 receptor agonist evoked Ca^{2+} mobilization in a high-throughput manner. Comparison of binding affinities of ligands in homogenates prepared from both cell lines revealed similar affinities for [^{3}H]CP55,940 displacement with the following rank order: CP55,940 ~ WIN55,212-2 > SR144528 > JWH015 ~ AM1241 ~ AM630 > SR141617A ~ AM251. In comparison at cannabinoid hCB_1 receptors: the rank order was: SR141617A ~ CP55,940 > AM251 > WIN55,212-2 > AM1241 ~ SR144528 > JWH015 ~ AM630. No significant differences in cannabinoid receptor agonist (CP55,940 ~ WIN55,212-2 > JWH015) or antagonist (SR144528 ~ AM1241 > AM630 > AM251 ~ SR141617A) profiles were observed in HEK-hCB_2-G_{qo5} and HEK-hCB_2-G_{qi5} cells as determined using intracellular Ca^{2+} measurements. Experiments with HEK-hCB_2-G_{qi5} cells carried out by investigating interactions among CP55,940, carbachol, thapsigargin, and U73122 revealed that the mechanism of cannabinoid hCB_2 receptor coupling via chimeric G proteins to Ca^{2+} mobilization involves phospholipase C-inositol trisphosphate (PLC-IP_3) and that it is less efficient in comparison to the endogenous muscarinic mediated PLC-IP_3-Ca^{2+} pathway. This study demonstrates that expressed cannabinoid CB_2 receptors couple equally well to $G\alpha_{qo5}$ and $G\alpha_{qi5}$ proteins and that receptor agonist or antagonist pharmacology is not influenced by the nature of these coupled G proteins when heterologously expressed.

1. Introduction

The effects of cannabinoids are mediated by two types of cannabinoid receptors: CB_1 and CB_2. Both receptors have been targeted as potential therapeutic targets. Cannabinoid CB_1 receptor antagonists are being considered for the management of obesity, and cannabinoid CB_1 receptor agonists are thought to provide benefit in the control of pain and for the relief of symptoms associated with multiple sclerosis or spinal cord injury, such as muscle spasm or spasticity, and for the prevention of nausea and emesis. Selective cannabinoid CB_2 receptor agonists have potential utilities as analgesic agents, immunomodulators, and in the management of liver diseases (Pertwee, 1999a; Lotersztajn et al., 2008; Cheng and Hitchcock, 2007). They offer benefit in comparison to non-selective cannabinoid ligands by limiting the undesirable side effects associated with cannabinoid CB_1 receptor activation such as: sedation, hypothermia, catalepsy, and inhibition of activity or impaired ambulation. At present, proof of concept validation of the cannabinoid CB_2 receptor agonist approach is limited to pre-clinical studies. Potent cannabinoid CB_2 selective receptor agonists with acceptable pharmacokinetic profile, *in vivo* efficacies, and wide separation from adverse effects are needed to further validate cannabinoid CB_2 receptor as a therapeutic target.

The cloning of cannabinoid CB_2 receptor has greatly accelerated the understanding of physiology and pharmacology of this receptor. Human cannabinoid CB_2 receptor was first cloned from promyelocytic leukemia line HL-60 in 1993 (Munro et al., 1993), followed by the cloning of mouse and rat cannabinoid CB_2 receptors (Shire et al., 1996). There is 80–90% nucleic acid identity among the different species of cannabinoid CB_2 receptors. In contrast, the cannabinoid CB_1 receptor was first cloned from rat brain (Matsuda et al., 1990) subsequently from human brain (Gerard et al., 1991) and mouse brain (Chakrabarti et al., 1995). There is ~44% amino acid identity between cannabinoid CB_1 and CB_2 receptors.

Cannabinoid CB_2 receptors have been recently found to be expressed in the brain although their function remains to be established (Van et al.,

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2005; Onaivi et al., 2006). The presence of cannabinoid CB_2 receptors in immune cells (macrophages, B cells, T cells, etc.) is well established and thought to regulate immune functions. For example, activation of cannabinoid CB_2 receptors on human tonsillar B cells with low concentrations of delta 9-tetrahydrocannabinol and two synthetic cannabinoids, CP55,940 and WIN55,212-2, has been reported to stimulate B-cell proliferation that was sensitive to a selective cannabinoid CB_2 receptor antagonist (Derocq et al., 1995). Immunosuppressive effects on lymphocyte and macrophage function with cannabinoids have been also reported, albeit at relatively high (micromolar) concentrations (Kaminski et al., 1992; Friedman et al., 1995). A role of cannabinoid CB_2 receptors in analgesia has been proposed recently. AM1241, a selective cannabinoid CB_2 receptor agonist, produced antinociception to thermal stimuli in rats that was sensitive to a selective cannabinoid CB_2 receptor antagonist, AM630, but not to a selective cannabinoid CB_1 receptor antagonist, AM251 (Malan et al., 2001). AM1241 was also effective in reversing tactile and thermal hypersensitivity produced by ligation of the L5 and L6 spinal nerves in rats (Ibrahim et al., 2003). Another selective cannabinoid CB_2 receptor agonist GW405833 [1-(2,3-Dichlorobenzoyl)-5-methoxy-2-methyl-(2-(morpholin-4-yl)ethyl)-1H-indole] inhibited carrageenan-induced inflammatory hypersensitivity (Clayton et al., 2002). The cellular mechanisms by which cannabinoid CB_2 receptor activation results in antinociception remain to be elucidated.

Studies on recombinant cannabinoid CB_1 and CB_2 receptors expressed heterologously in mammalian (e.g. CHO, HEK-293) and non-mammalian cell lines (e.g. Sf9) as well as on cell types or tissues endogenously expressing cannabinoid receptors have revealed that both types of cannabinoid receptors are negatively coupled to adenylyl cyclase via type i/o guanine nucleotide binding ($G_{i/o}$) proteins (reviewed in Howlett et al., 2002; Pertwee, 1997). Another signal transduction mechanism that has been reported for cannabinoid receptors occurs via mitogen-activated protein kinase (MAPK) (Pertwee, 1999b; Bouaboula et al., 1995). In addition, cannabinoid CB_1 but not CB_2 receptors can also activate adenylyl cyclase via G_s protein, and inhibit Ca^{2+} and activate K^+ channels (reviewed in Howlett et al., 2002; Howlett, 2002).

Pharmacological characterization of novel cannabinoid receptor compounds usually involves radioligand binding experiments with high affinity non-selective cannabinoid ligands, [^{3}H]CP55,940 and [^{3}H]HU243, moderately cannabinoid CB_2 selective, [^{3}H]WIN55,212-2, and cannabinoid CB_1 receptor selective [^{3}H]SR141716A (reviewed in Pertwee, 1997, 1999b). Although radioligand binding studies provide useful information about ligand affinities including relative selectivity, such studies provide no functional information. At present, available tools to investigate the functional properties at cannabinoid CB_2 or CB_1 receptors primarily involve adenylyl cyclase, GTPγS, and MAPK assays (reviewed in Pertwee, 1997, 1999b). However, all of these methodologies have inherent limitations. For example, adenylyl cyclase and MAPK assays most commonly rely on the detection of cAMP levels or phosphorylated MAPK with specific antibodies that are prone to variability of the detection reagents and can have low throughput. GTP hydrolysis and adenylyl cyclase assays involve the use of radioactive GTPγ[^{35}S] and [^{125}I]-labeled cAMP recognizing antibodies that require proper and expensive disposal procedures as well as present potential radioactivity exposure risks, especially when utilized in high throughput formats.

Recent developments in chimeric G protein technology have provided another alternative for the pharmacological study of G-protein coupled receptors, especially for those that are linked to adenylyl cyclase. Conklin and Bourne (1993) and Conklin et al. (1993) have shown that chimeric G proteins in which the last five carboxyl-terminal amino acids of the $G\alpha_q$ protein were replaced with corresponding sequences of either $G\alpha_i$ or $G\alpha_o$ proteins link the activation of the chimeric G proteins to the phospholipase C-inositol trisphosphate-Ca^{2+} (PLC-IP_3-Ca^{2+}) pathway. More importantly, this leads to mobilization of intracellular Ca^{2+} levels that can be measured with high-throughput Ca^{2+} imaging methods. So far, this technology has been successfully documented for at least 18 G protein coupled receptors with the inclusion of coupling via promiscuous G proteins (i.e. $G\alpha_{15}$ and $G\alpha_{16}$) (Coward et al., 1999). Cannabinoid receptors have been also successfully linked to mobilization of intracellular Ca^{2+} via chimeric G proteins by two separate approaches. One involved generation of a polypeptide linking the cannabinoid CB_1 receptor to promiscuous $G\alpha_{16}$ (Martin et al., 2002) and the other co-expression of cannabinoid CB_2 and chimeric $G\alpha_{qo5}$ protein (Mukherjee et al., 2004). Another group, although did not study the effects on intracellular Ca^{2+}, showed increases in inositol phosphate formation when the cannabinoid CB_2 receptor activation was coupled to $G\alpha_{16/Z}$ protein (New and Wong, 2003). These studies, hence, show that the coupling of cannabinoid CB_2 receptors via chimeric or promiscuous G proteins to mobilization of intracellular Ca^{2+} is possible.

A single G-protein coupled receptor may exist in multiple active conformations and couple differentially to specific G proteins and intracellular second messenger effector systems (Kenakin, 2002a,b). For example, CHO cells expressing 5-HT_{2A} and 5-HT_{2C} receptors showed agonist specific responses in phospholipase C-dependent inositol phosphate accumulation and phospholipase A_2 specific arachidonic acid release (Berg et al., 1998). The coupling of β_{3a}- and β_{3b}-adrenergic receptors to $G\alpha_{qi}$ was less efficient than to $G\alpha_{qs}$ suggesting preference for a given G protein mediated pathway (Lenard et al., 2006). Cannabinoid CB_2 receptors likely couple to multiple G proteins as well. In HL-60 cells expressing cannabinoid CB_2 receptors, CP55,940 application decreased the expression of at least 3 different $G\alpha_i$ proteins (Bouaboula et al., 1999). Cannabinoid CB_2 receptors when expressed in insect Sf9 cells interacted more efficiently with $G\alpha_i$ than $G\alpha_o$ proteins as measured by GTPγ[^{35}S] binding (Glass and Northup, 1999) suggesting cannabinoid CB_2 receptor–G protein specific coupling. Furthermore, agonist-specific rank order of potencies and fractional occupancies for intracellular effectors (MAPK, adenylyl cyclase, and Ca^{2+} mobilization) were noted for cannabinoid CB_2 receptors studied in CHO cells (Shoemaker et al., 2005).

Accordingly, this study aimed to compare the efficiencies of coupling of cannabinoid CB_2 receptors via $G\alpha_i$ and $G\alpha_o$ proteins by co-expressing cannabinoid hCB_2 receptors with chimeric $G\alpha_{qo5}$ or $G\alpha_{qi5}$ proteins and studying the affinities to cannabinoid ligands using radioligand binding and functional responses to receptor agonists and antagonists utilizing Ca^{2+} imaging. Furthermore, evidence is provided that mobilization of Ca^{2+} in chimeric $G\alpha_{qi5}$ cells co-expressing expressing cannabinoid hCB_2 receptor is mediated by phospholipase C pathway and that it involves a subset of IP_3 sensitive Ca^{2+} stores.

2. Materials and methods

2.1. Cell lines and cell culture

Previous reports from our group provide details of the generation and maintenance of the HEK-293 stable cell lines expressing cannabinoid hCB_2 receptor only (HEK-hCB_2) and co-expressing cannabinoid hCB_2 receptor and $G\alpha_{qo5}$ protein (HEK-hCB_2-G_{qo5} cells) (Mukherjee et al., 2004; Yao et al., 2006). In this study, the stable cell line co-expressing cannabinoid hCB_2 and $G\alpha_{qi5}$ (HEK-hCB_2-G_{qi5} cells) was obtained by transfecting HEK-hCB_2 cells with HA-tagged $G\alpha_{qi5}$ DNA clone (Molecular Devices, Sunnyvale, CA, USA) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, HEK-hCB_2 cells were grown to confluency and exposed to DNA-lipofectamine 2000 reagent complexes. Forty-eight hours after transfection, the cells were split and grown as single colonies under antibiotic selection (25 μg/ml zeocin and 200 μg/ml hygromycin B, Invitrogen). Individual clones were functionally screened using a Ca^{2+} mobilization assay in a FLIPR® instrument (Molecular Devices, see methods below). The clonal cell lines were maintained in Dulbecco's modified Eagle's medium (Gibco-BRL,