Review

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GPR55 – a putative “type 3” cannabinoid receptor in inflammation

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Abstract: G protein-coupled receptor 55 (GPR55) shares numerous cannabinoid ligands with CB1 and CB2 receptors despite low homology with those classical cannabinoid receptors. The pharmacology of GPR55 is not yet fully elucidated; however, GPR55 utilizes a different signaling system and downstream cascade associated with the receptor. Therefore, GPR55 has emerged as a putative “type 3” cannabinoid receptor, establishing a novel class of cannabinoid receptor. Furthermore, the recent evidence of GPR55-CB1 and GPR55-CB2 heteromerization along with its broad distribution from central nervous system to peripheries suggests the importance of GPR55 in various cellular processes and pathologies and as a potential therapeutic target in inflammation.

Keywords: CB2; endocannabinoid system; GPR55; immune modulation; inflammation; sepsis.

Introduction

The endocannabinoid system (ECS) has a tremendous potential for experimental and clinical research, based on their ubiquitous presence in vertebrates and role in fundamental physiological and pathological processes, including pain modulation, immune function, neuroprotection, cancer, cardiovascular diseases, fertility and appetite [1]. Clinical studies using cannabinoid receptor agonists and antagonists have suggested their possible use in modulating acute and/or chronic diseases, e.g. hypertension, glaucoma, emesis, anxiety, depression, gastrointestinal and hepatic disorders, ulcerative colitis and neuropathic and inflammatory pain [2, 3].

The evidence for homeostatic and protective functions of endocannabinoids in inflammation encourages studies of ECS as a therapeutic target in sepsis. Further elucidation of endocannabinoid signaling pathways will aid in the design of compounds with site specificity and high affinity to various receptor targets [4, 5]. In this review, current knowledge of the expression and function of the orphan G protein-coupled receptor 55 (GPR55), a putative “CB3” receptor, is summarized, highlighting its potential to be a therapeutic target in inflammation.

Endocannabinoid system

The marijuana plant, Cannabis sativa, has been used for more than 4000 years for a variety of purposes from medicine to recreation owing to their psychoactive properties. There are over 80 active phytocannabinoids in the marijuana plant, which exert their effects on the central and peripheral nervous system by binding and activating specific receptors in the cell membrane [6]. The most prominent and best-investigated phytocannabinoids from C. sativa are Δ⁹-tetrahydrocannabinol (THC) and cannabidiol [7]. Although these phytocannabinoids have been studied extensively for their psychoactive properties, their potential use as therapeutic agents have been under mined until the discovery of the ECS in the early 1990s [6].

The ECS consists of three components: endogenous lipid-signaling molecules (endocannabinoids), G protein-coupled cannabinoid receptors and the enzymes for biosynthesis and degradation. Endocannabinoids are endogenous agonists of cannabinoid receptors and are found to mimic the pharmacological actions of the phytocannabinoids [6, 8]. The first identified endocannabinoid was N-arachidonyl ethanolamine (anandamide or AEA) in...
1992, followed by 2-arachidonyl (2-AG) in 1995, which are the best-studied endocannabinoids to date [9, 10].

Biosynthesis of endocannabinoids takes place on demand either by activity-dependent or receptor-stimulated cleavage of arachidonic acid in the cell membrane, which activates enzymes including N-acetylphosphatidylethanolamine-hydrolyzing phospholipase D for AEA synthesis and two isoforms of diacylglycerol lipases (DAGLα and DAGLβ) for 2-AG synthesis [11, 12]. Because of the lipophilic nature of endocannabinoids, they are not suitable for storage in vesicles and thus are released immediately after their production. Followed by the biosynthesis, these lipid transmitters then locally activate cannabinoid receptors in response to different physiological and pathological stimuli [5].

Endocannabinoids are degraded by enzymatic hydrolysis mediated by specific intracellular enzymes to limit excessive endocannabinoid signaling. The major degradation enzymes responsible for these reactions have been identified as fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), for AEA and 2-AG, respectively [13, 14]. To inhibit the degradation of endocannabinoids, enzyme inhibitors are developed. The most well-characterized inhibitors of degradation enzymes are URB597 and JZL184 for inhibiting FAAH and MAGL degradation, respectively [15, 16].

The cannabinoid receptors are members of seven-transmembrane spanning class A G protein-coupled receptor (GPCR) subfamily. In the late 1980s and early 1990s, two high-affinity cannabinoid receptors that bind anandamide and 2-AG have been identified by molecular cloning: cannabinoid receptor type 1 and type 2 (CB1 and CB2) [17–19]. CB1 receptors are predominantly expressed in the central nervous system (CNS) and mediate most psychoactive effects, whereas CB2 receptors are mainly distributed in peripheral and immune cells, mediating immunosuppressive effects [19–21]. When activated by endocannabinoids, both CB1 and CB2 receptors are coupled to Gαi/o heteromeric G protein and thereby inhibit adenyl cyclase, activate mitogen-activated protein kinases and further turn on numerous transcription factors [5].

The discovery of cannabinoid receptors is followed by development of selective CB1 and CB2 receptor agonists and antagonists [22]. The best known of CB1-selective agonists include R-(+)-methanandamide, arachidonoyl-Z-chloremethylamide, arachidonyl-cyclopropylamide and O-1812, and antagonists are AM251 and AM281. As for CB2-selective receptors, there are JWH133, HU308 and AM1241 for agonists and SR144528 and AM630 for antagonists [6]. Compounds such as (–)11-hydroxy-Δ8-THC-dimethylheptyl (HU-210), CP55940, and R-(+)-WIN55212 are able to activate both CB1 and CB2 receptors [6].

GPR55 expression and signaling

In the last decade, accumulating evidence from studies using either selective CB1 and CB2 ligands or CB1 and/or CB2 knockout mice has suggested the existence of one or more additional cannabinoid receptors distinct from CB1 and CB2 receptors [23]. Recently, the orphan GPR55 showed binding with some cannabinoids and non-cannabinoid ligands and therefore presented as the main candidate to be considered as the ‘‘third’’ cannabinoid receptor [24].

In 1999, Sawzdargo et al. first isolated and cloned human GPR55 (hGPR55) with high expression in the regions of the CNS including the hippocampus, caudate, putamen, hypothalamus, cerebellum, thalamus andpons. It is also expressed in peripheral tissues such as the endothelial cells, adrenal glands and gastrointestinal tract [24, 25]. More recently, high expression of GPR55 is also found on lymphocytes and spleen, as well as on many cancer cells, which correlates with the rate of cancer cell proliferation [26, 27]. Although GPR55 shares several cannabinoid ligands with CB1 and CB2, GPR55 exhibits a low amino acid identity to CB1 (13.5%) and CB2 (14.4%). However, GPR55 is clearly a member of class A GPCRs, based on amino acid homology with P2Y5 purinergic receptor (29%), GPR23 (30%) and GPR35 (27%) [25].

The endocannabinoid receptor signaling pathways are not yet fully elucidated. The classical CB1 and CB2 signaling are known to utilize primarily Goαi/o heteromeric proteins and adenylate cyclase to activate multiple different downstream cascades. However, it is currently found that the signal transduction pathway of GPR55 differs from that of CB1 and CB2. It has been found that GPR55 only couples to Goαi2,3 proteins, thereby activating ras homologue gene family member A (RhoA) and Rho-associated protein kinase (ROCK). Activation of RhoA and ROCK elicits phospholipase C (PLC) pathway to increase intracellular Ca2+, activates small GTPase proteins rhoA, Rac and cdc42, and turns on extracellular regulated kinase (ERK) phosphorylation (Figure 1) [24, 28, 29].

GPR55 pharmacology

Despite the low homology with the classical cannabinoid receptors (CB1 and CB2), several cannabinoids are found
to interact with GPR55, including the cannabinoid agonist, Δ⁹-THC, CB1-selective antagonist/inverse agonist rimonabant and CB1 and CB2 agonist CP55, 940 [30]. It has been shown that 1-lysophosphatidylinositol (LPI), O-1602 and AM251 have agonist effects for GPR55, whereas SR141716A (Rimonabant), O-1918 and cannabidiol (CBD) have antagonist effects [31–33]. However, there is controversial evidence regarding the opposite or divergent pharmacological effects through GPR55 compared to CB receptors [34]. For instance, anandamide and 2-AG are reported to activate GPR55, while other groups failed to detect the agonist effect by these cannabinoids [29, 34, 35].

Most recent studies on receptor coupling and heteromer formation present an explanation of the aforementioned controversial findings on GPR55 ligands [33, 36, 37]. Inconsistent activities of GPR55 is observed in response to cannabinoid ligands, influenced by the assay used to assess receptor function [33]. With that regard, a novel label-free assay is evaluated and successfully detected agonist activity for all GPR55 tested [33]. Furthermore, it is reported that CB1 and other class A GPCRs could form homomers and heteromers, which alter the biochemical property of the receptors. Naturally co-expressed receptors are present in several cell types. For example, GPR55 and CB2 are co-expressed on neutrophils and cancer cells [37], and GPR55 and CB1 are co-expressed in several brain regions [36]. In these co-expression assays, GPR55-mediated signaling is inhibited with inactive CB1; in contrast, CB1-mediated signaling is enhanced with GPR55 [36]. On the other hand, the activation of GPR55 heteromers with CB2 receptor in cancer cells is ligand concentration dependent [37]. Furthermore, GPR55-CB2 heteromers are shown to elicit different pathways via ligand- and concentration-specific crosstalk (Figure 2) [38]. Overall, GPR55 heteromers with either CB1 or CB2 exhibit function as novel signaling entities [36, 37].

![Figure 1: Overview of the GPR55 signaling pathway by LPI. Extracellular LPI can bind and activate GPR55. Activation of GPR55 is coupled to \( \text{G}_{12,13} \) proteins, thereby activating ras homologue gene family member A (RhoA) and ROCK. ROCK then turns on PLC pathway, which allows cytosolic concentration of \( \text{Ca}^{2+} \) to increase. Increased intracellular \( \text{Ca}^{2+} \) in turn leads to ERK phosphorylation and gene expression [24, 28, 29].](image1)

![Figure 2: Schematic of signaling modulation of GPR55-CB2 heteromer. (A) CB2 signaling pathway is inhibited in the heteromer when both GPR55 and CB2 are activated by the agonists (negative crosstalk). (B) GPR55 antagonist inhibits CB2 agonist-induced heteromer activation (left) or CB2 antagonist inhibits GPR55 agonist-induced heteromer activation (right, cross antagonism) [37, 38].](image2)
GPR55 in inflammation

GPR55 is widely distributed throughout the body, and its role has been suggested to be involved in many physiological and pathophysiological processes, including the role in pathophysiology of the gut [39–41], inflammatory and neuropathic pain [42] and modulation of innate and adaptive immune system [43–45].

As GPR55 is expressed throughout the rodent gastrointestinal tract, many gastrointestinal disease models were adopted to reveal the role of GPR55 in intestinal inflammation. For example, increased expression of GPR55 is observed in lipopolysaccharide (LPS)-treated intestine [41] in vivo suggesting GPR55 involvement in intestinal inflammation. In addition, GPR55 knockout mice showed less severe colitis compared to CB1 or CB2 knockout mice, suggesting pro-inflammatory role of GPR55 in experimental colitis induced by dextran sulfate sodium [39].

In another experimental colitis model, an endogenous anandamide-related lipid, palmitoylethanolamide (PEA), is shown to reduce neuro-inflammation and chronic pain in the colitis induced by intracolonic administration of dinitrobenzenesulfonic acid [46]. In this study, mRNA expression of GPR55 is significantly increased compared to CB2 when PEA is administered [46]. Furthermore, in a mechanical hyperalgesia model using Freund’s complete adjuvant, inflammation and neuropathic hypersensitivity are absent in GPR55 knockout mice, suggesting a pro-inflammatory role of GPR55 [42].

While the role of GPR55 was not fully elucidated, CB2R has been thought to be responsible for activation and recruitment of neutrophils as well as regulation of other immune cells. Recently, Balenga and colleagues found that GPR55 is involved in neutrophil chemotaxis and recruitment via crosstalk with CB2R orchestrated by various chemoattractants in downstream signaling pathway [43]. The interplay between GPR55 and CB2R during inflammation enhances neutrophil migration efficiency and revokes degranulation and ROS formation in neutrophils [43]. Moreover, GPR55 found on mast cells exerts its anti-inflammatory effect by inhibiting mast cell-mediated release of nerve growth factor and attenuating angiogenesis [44]. Lastly, it has been reported that GPR55 is highly expressed on monocytes and natural killer (NK) cells, compared to several innate and adaptive immune cells from human peripheral blood mononuclear cells [45]. Activation of LPS-activated monocytes and NK cells by GPR55 shows increase in pro-inflammatory cytokines, cytolytic activity of NK cells and decrease in endocytic activity of monocytes [45]. Altogether, these evidences remark on the potential role of GPR55 in innate immunity modulation and inflammation. Therefore, GPR55 is a novel candidate for treatment of inflammatory diseases.

Conclusions

The ECS is under investigation as an attractive target of drug development for inflammatory diseases. Cannabinoid receptor expression in different tissues and cells, along with their high affinity to bind cannabinoid ligands, contributes to their ability to modulate numerous cellular processes and pathologies.

GPR55, a putative “type 3” cannabinoid receptor, triggers distinct signaling pathways in response to inflammatory mediators. Although recent studies have rapidly expanded G protein-coupled receptor pharmacology and pathophysiology, more research for selective agonists and antagonists is required to resolve the controversial pharmacology of GPR55. In addition, biochemical insights in the receptor coupling and in vivo studies in knock-out mice will aid to further elucidate GPR55 mechanism of action and to develop specific GPR55 agonists and antagonists for the use in inflammation.

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