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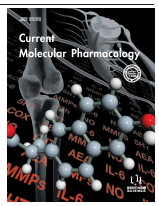
REVIEW ARTICLE

New Insights in Cannabinoid Receptor Structure and Signaling



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Abstract: ***Background:*** Cannabinoid has long been used for medicinal purposes. Cannabinoid signaling has been considered the therapeutic target for treating pain, addiction, obesity, inflammation, and other diseases. Recent studies have suggested that in addition to CB1 and CB2, there are non-CB1 and non-CB2 cannabinoid-related orphan GPCRs including GPR18, GPR55, and GPR119. In addition, CB1 and CB2 display allosteric binding and biased signaling, revealing correlations between biased signaling and functional outcomes. Interestingly, new investigations have indicated that CB1 is functionally present within the mitochondria of striated and heart muscles directly regulating intramitochondrial signaling and respiration.

Conclusion: In this review, we summarize the recent progress in cannabinoid-related orphan GPCRs, CB1/CB2 structure, Gi/Gs coupling, allosteric ligands and biased signaling, and mitochondria-localized CB1, and discuss the future promise of this research.

Keywords: Cannabinoid receptor, structure, orphan GPCRs, allosteric ligand, biased signaling, mitochondria.

1. INTRODUCTION

Cannabis sativa L., commonly known as marijuana, has been used in different civilizations for a variety of medical applications such as appetite stimulation and the treatment of pain, nausea, fever, and gynecological disorders for thousands of years [1, 2]. There are more than 100 different cannabinoids isolated from cannabis plant, exhibiting varied effects by activating cannabinoid receptors. The most notable cannabinoid is Δ9-tetrahydrocannabinol (THC), the principal psychoactive constituent in cannabis. Cannabidiol (CBD) is another major constituent. Apart from the phytocannabinoids, ligands for cannabinoid receptors also include the endocannabinoids that are produced naturally throughout the body and the synthetic cannabinoids [3]. There are six recognized endocannabinoids currently. Anandamide (AEA) was the first one identified [4]. 2-arachidonoyl glycerol (2-AG), N-arachidonoyl-dopamine (NADA), 2-arachidonyl glyceryl ether (noladin ether), virodhamine (OAE) as well as Lysophosphatidylinositol (LPI) were subsequently discovered [5, 6].

The mechanism of action of cannabinoid drugs became clear with the subsequent identification of two cannabinoid receptors, termed CB1 and CB2 [7, 8]. They are members of the class A G protein-coupled receptor (GPCR) family and possess approximately 44% amino acid similarity [9]. As shown in Fig. (**1**), both receptors share a common feature of class A GPCRs, possessing a glycosylated extracellular amino-terminal (N-term) and an intracellular carboxyl-terminal (C-term) domain connected by seven transmembrane domains (7TM), three extracellular loops (ECL1, ECL2, and ECL3) and three intracellular loops (ICL1, ICL2 and ICL3).

CB1 receptors are predominantly expressed in the brain, particularly in cerebral cortex, hippocampus, basal ganglia, and cerebellum [7, 10], where they mediate the majority of the psychotropic and behavioral effects of cannabis. Recent evidence has suggested that CB1 are also expressed in several peripheral tissues [11, 12], including the spleen, lung, thymus, heart, and vasculature. In comparison, CB2 receptors are abundantly expressed in peripheral tissues with immune function, such as leukocytes, spleen, tonsils, thymus, as well as the lung and testes [9, 13, 14]. Subsequent studies have shown the role of the CB2 receptors in a variety of systems including the central nervous systems (CNSs), as well as the cardiovascular and respiratory systems, bone, the gastrointestinal (GI) tract, liver and the reproductive system [15-17]. As shown in Fig. (**1**), existing evidence demonstrated that both CB1 and CB2 receptors are coupled to Gi/o protein to inhibit adenylyl cyclase activity leading to a decrease of cAMP levels. Alternatively, CB1 receptors have also been shown to be capable of coupling to Gs protein stimulating cAMP production in some cases [18-20]. It has been reported that a cannabinoid-mediated the increase of cAMP in

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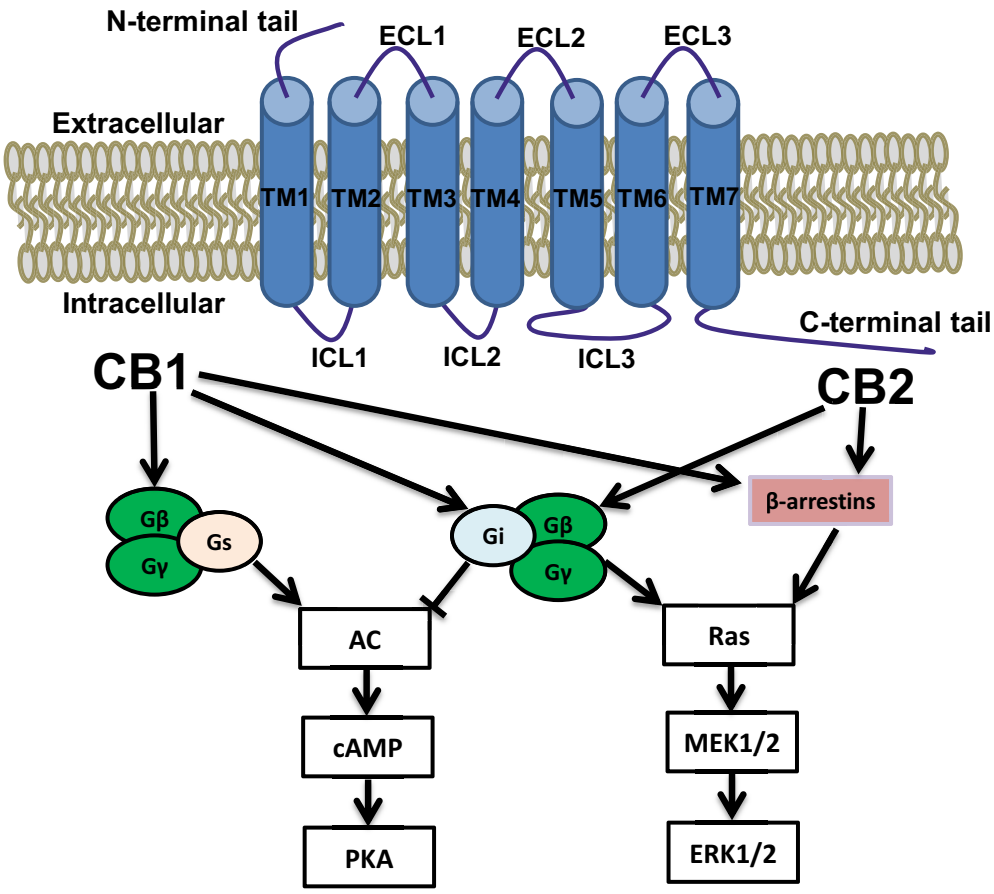

Fig. (1). Schematic model of CB1 and CB2, showing the terminal tails, TM helices, intracellular loops, as well as the major intracellular signaling pathways.

cultured rat striatal neurons as well as in CB1-expressing CHO cells in the presence of forskolin [14]. The interaction of the CB1 receptor with Gs has also been confirmed in CHO cells transfected with recombinant human CB1 receptors [18, 19]. However, it is not clear whether CB2 receptor could bind with other G proteins [21]. The dual inhibition and activation effects of the CB1 receptor on adenylyl cyclase were verified to be ligand-specific [18, 22]. Our recent studies have revealed that the intracellular loop 2 (ICL2) of both CB1 and CB2 receptors and in particular the residue Leu-222, which resides within a highly conserved DRY(X)5PL motif, played a critical role in Gs and Gi protein coupling and specificity [14, 21].

The cannabinoid receptors have always been the targets of intensive drug development efforts. Accumulating studies suggest that the pharmacologic regulation of the CB1 has been proposed as a promising therapeutic strategy for a wide range of disorders, including pain, inflammation, obesity, neurodegenerative disorders, and cancer [10, 12, 23, 24]. Meanwhile, CB2, predominantly expressed in the peripheral areas, is an attractive therapeutic target for immune-modulators, pain management, osteoporosis, and the treatment of liver diseases [25-27]. This chapter will provide an overview of the recent studies regarding the structure and allosteric modulation of cannabinoid receptors, CB1 and CB2, and characterization of orphan cannabinoid receptors. We also highlight the most recent advances in the biased signaling and intracellular localization of cannabinoid receptors. These findings uncover a previously unexplored framework for cannabinoid system-targeted drug discovery.

2. CANNABINOID RECEPTOR STRUCTURE

The human cannabinoid system plays critical roles in the regulation of human physiology and makes up the targets for intensive drug discovery efforts. On the other hand, the elucidation of the detailed structure of the cannabinoid receptors will facilitate a better understanding of how agonists and antagonists engage to modulate the downstream signaling of the cannabinoid system. Therefore, the human cannabinoid system becomes an attractive area of research. In addition to the improvement of methods including recombinant expression systems and protein engineering, the breakthroughs in key techniques such as the use of a T4 lysozyme insertion, lipidic cubic phase crystallization and microfocus diffraction beamlines lead to a remarkable progress in the elucidation of GPCR structures [28, 29].

The first 2.8 Å crystal structure of the human CB1 receptor in complex with a tight binding AM6538, a stabilizing antagonist, was successfully resolved in 2016 [30]. The structure of the CB1-AM6538 complex reveals key characteristics of the receptor and decisive interactions for antagonist binding. The N terminus of CB1 plays a key role in ligand recognition, which is the same as other receptors.

Specifically, the non-truncated part of the N terminus of CB1 forms a V-shaped loop that inserts into the ligand-binding pocket and acts as a plug to restrict access to the pocket from the extracellular side. Meanwhile, the extracellular loop 2 (ECL2) composed of 21 residues folds into a complex structure, projecting four residues (268-271) into the binding pocket. It has been reported that the four residues are essential for mediating interactions with certain kinds of ligands [31] and the two cysteines (Cys257 and Cys264) in ECL2 are pivotal to the function of CB1 [32]. Two months later, the structure of the human CB1 receptor bound to the inhibitor taranabant at 2.6-Å resolution was determined [33]. Consistent with the AM6538-bound structure, the extracellular surface of CB1, including the highly conserved membrane-proximal N-terminal region, forms a critical part of the ligand-binding pocket. However, the taranabant-bound structure showed a stronger electron density for the ligand and the N-terminal region than the former one. It may be important for functional interpretation and prediction.

The structures of CB1 complexed with the antagonist AM6538 and taranabant reveal a molecular understanding of the inactivation state of the receptor, yet they do not show how CB1 elicits its diverse physiological effects. For this reason，two agonist-bound crystal structures of human CB1 in complex with a tetrahydrocannabinol (AM11542) and a hexahydrocannabinol (AM841) at 2.80 Å and 2.95 Å resolution were determined [34]. Comparisons between the agonist- and antagonist-bound CB1 complexes reveal notable structural rearrangements. A notable feature of the CB1 agonist bound structure seems to use an extended molecular toggle switch termed 'twin toggle switch', involving a synergistic conformational change between Phe2003.36 and Trp3566.48. In addition, the two CB1-agonist complexes reveal a large (53%) reduction in the volume of the ligand-binding pocket, and subsequent increase in the surface area of the G-protein-binding region. Such plasticity in the orthosteric binding pocket enables CB1 to respond to a variety of ligands with different sizes and shapes.

During the past decade, more than 40 unique receptors have been characterized by X-ray crystallography [28, 35]. However, the atomic level details regarding the CB2 receptor activation remain unanswered. Several research groups tried to construct homology-based comparative CB2 models using some 3D crystal structures of GPCRs, like rhodopsin, β2AR, and A2AAR. The first 3D homology model for the CB2 was reported in 1999, based on the α-helical periodicity in the CB2 sequence [36]. A comparative 3D CB2 model was constructed in 2003 [37]. Three years later, other CB2 comparative models were clarified based on the crystal structure of bovine rhodopsin determined at 2.8 Å as a template, and those models represented the inactivated state [38-40]. During 2011 and 2012, newer 3D CB2 models based on A2AAR were generated [41, 42]. All models above were used to analyze CB2 ligand binding properties and explain the effects of individual biological or pharmacological experiments. However, they are limited to the few crystal structures low sequence identities to CB2.

Although most of CB2 models are built on the reported inactive GPCRs, Xie *et al*. constructed both active as well as the inactive CB2 models by homology modeling. The active CB2 bound with both the agonist and G protein, while the inactive CB2 bound with inverse agonist [43]. In these models, the inactive CB2 and the inverse agonist remained stable during the molecular dynamics (MD) simulation. While during the assay, dynamical details about the breakdown of the "ionic lock" between R1313.50 and D2406.30 as well as the outward/inward movements of transmembrane (TM) domains that bind with G proteins and agonist (TM5, TM6, and TM7) were observed. Moreover, W2586.48 in TM6 and residues in TM4 (V1644.56−L1694.61) contribute greatly to the binding of the agonist on the basis of the binding energy decomposition, while residues S180-F183 in ECL2 may be essential in recognition of the inverse agonist. Taken together, the increasingly refined homology-based CB2 models provide new insights to a better understanding of the structure and conformation of CB2, useful for virtual screening and drug design.

3. CANNABINOID-RELATED ORPHAN GPCRS

Up to date, there are still around 100 GPCRs with no known endogenous ligand(s), termed orphan GPCRs (oGPCRs) [44]. The deorphanization of oGPCRs opens new possibilities in GPCR-targeted drug discovery. Using $CB1^{-/-}$ and $CB2^{-/-}$ mice, previous studies have suggested the existence of putative orphan cannabinoid receptors, the so-called non-CB1/CB2 receptors, in the vasculature, central nervous system and immune cells [45, 46]. Three oGPCRs, GPR55, GPR18 and GPR119, have emerged as putative non-CB1/CB2 receptors [47, 48].

GPR55 is an orphan GPCR in the purinergic subfamily. GPR55 was first identified from in silico studies and later cloned in 1999 [49]. The first report linking GPR55 to cannabinoids appeared in a patent from GlaxoSmithKline [50]. Subsequently, AstraZeneca published a patent confirmed the association of GPR55 with cannabinoids [51]. However, GPR55 has very low homology to the classical cannabinoid receptors (about 14% and 15% with CB1 and CB2 respectively) [52]. The endogenous ligand of GPR55 L-α- lysophosphatidylinositol (LPI), was first identified by Oka *et al* [52]. Recently, accumulating data suggest that some synthetic cannabinoid ligands can also activate GPR55, such as CB2 agonists HU210 [53] and CB1 antagonists AM251 and rimonabant [54]. However, in some studies, rimonabant also acted as a GPR55 antagonist [55]. Several groups have pharmacologically determined the downstream signaling of GPR55 in HEK293 cells. In their studies, the GPR55-mediated activation of ERK1/2 signaling [52], Rho activation, calcium ion elevations, and the activation of transcription factors, including nuclear factor of activated T-cells and cAMP response element binding protein (CREB) [56] were characterized. Additionally, GPR55-mediated β-arrestin recruitment has also been demonstrated [57]. Interestingly, GPR55 has been shown to form heteromers with CB2 receptor, which impacts on cell signaling [58], leading to enhanced MAP kinase activation, and reduced transcription factor generation [59].

GPR18 is less homologous to CB1 and CB2 (~13% and 8%), but GPR18 is closely related to the endocannabinoid system (ECS). A number of cannabinoid ligands have been described to be active as agonists or antagonists, such as abn-

CBD, O1602, Δ9-THC and N-arachidonoylcyclopropyl amide have all been described as full agonists in GPR18-transfected HEK cells [60]. Several studies have suggested that N-arachidonylglycine (NAGly) is an endogenous ligand for GPR18. However, NAGly has no activity on the classical cannabinoid receptors CB1 and CB2 [61]. Therefore, it is difficult to determine whether GPR18 is a cannabinoid receptor.

GPR119 is an orphan receptor originally identified in genome-sequencing efforts and expressed predominantly in the pancreas and gastrointestinal tract. Many types of research show that GPR119 regulates energy balance in the pancreas [62]. In 2006, Overton *et al*. identified GPR119 as a cannabinoid receptor [48], and it can be activated by oleoylethanolamine (OEA; the first identified endocannabinoid). Subsequently, 2AG has also been identified as a GPR119 agonist [63]. A third endogenous GPR119 activator, oleoyllysophosphatidylcholine, has also been identified with some structural commonality with the endogenous ligand of GPR55 [64]. In addition, there are many GPR119 small molecule agonists, for example PSN75963 evokes concentration-dependent increases, in cAMP in GPR119-transfected cells by coupling with Gas [48]. AS1269574 is a structurally distinct synthetic GPR119 agonist, which was identified to enhance glucose-stimulated insulin secretion both *in vitro* and *in vivo*. To date, no antagonists of GPR119 have been described, this is may be due to the fact that there appears to be little therapeutic potential for them [47]. Studies have shown that GPR119 may be involved in the regulation of type 2-diabetes, metabolic disorders and obesity. In fact, synthetic GPR119 agonists showed positive results in phase II clinical trials of type 2 diabetes [58].

Because of their close phylogenetic relationship with the cannabinoid receptors or their ability to interact with lipids, the orphan receptors GPR3, GPR6, GPR12, GPR23, and GPR92 have been categorized as possible cannabinoid receptor candidates [65, 66]. Orphan receptors GPR3, GPR6, and GPR12 share over 60% of sequence similarity, and are mainly expressed in the brain and the reproductive system [67]. Among them, both GPR3 and GPR6 have been recently found to be activated by the nonpsychoactive phytocannabinoid cannabidiol (CBD) [68]. Using the β -arrestin PathHunter assay system，*N*-arachidonoyl glycine (NAGly) has been shown to elicit a weak activation of GPR92 [69]. However, to date, there are no published data to show that any known agonists or antagonists for CB1 or CB2 receptors are able to activate orphan receptors GPR12 and GPR23. Therefore, there is a need for these orphan receptors to be examined for their responsiveness to different endogenous, phytogenic, and/or synthetic cannabinoid agonists and antagonists.

4. ALLOSTERIC MODULATORS OF CANNABINOID RECEPTORS

Allosteric ligands, binding to the secondary binding sites, are divided into three groups according to their effects on orthosteric ligand (bind to the same site as the endogenous ligand) responses: Positive Allosteric Modulators (PAMs), Negative Allosteric Modulators (NAMs), and Neutral Allosteric Ligands (NALs) [70, 71]. Pharmacological advantages such as higher specificity and thus reduced side effects have motivated efforts to characterize and develop both positive and negative allosteric modulators targeting GPCR in academia and industry. Here we list both the CB1 and CB2 allosteric modulators.

It has been validated that significant abuse potential and the risk of psychological and mood-altering side effects seriously affect the development of orthosteric agonists and antagonists targeted CB1 [72-74]. This has prompted academia and industry to develop both positive and negative CB1-targeted allosteric small-molecules. Since the first report on a CB1 allosteric ligand-binding site in 2005 [75], both PAM such as PAM1, ZCZ011, Lipoxin A4 and RTI-371 and NAM including Org27569, ABD1027, PSNCBAM-1, GAT358, Pregnenolone, CBD and Fenofibrate, have been developed [76]. The synthetic indole Org27569 is one of the most intensively studied CB1 allosteric modulators. The initial characterization showed that Org27569 displayed contradictory pharmacological behavior as PAMs of orthosteric agonist affinity, but NAMs of agonist efficacy [75-77]. Further profiling demonstrated that Org27569 acts as a biased allosteric agonist showing the potential to inhibit agonist-induced G-protein-mediated inhibition of cAMP production, but to enhance agonist-induced ERK1/2 activation [78]. To date, a number of Org27569 analogs have been developed [79, 80]. Among them, one compound was found to specifically induce β -arrestin1-mediated pathway-biased signaling [81]. Interestingly, the nonpsychoactive cannabidiol (CBD), a phytocannabinoid compound with the potential to interact with many nonendocannabinoid signaling targets including the opioid receptors, the serotonin 5HT1A receptors, the PPARγ receptors, and the transient channel receptor[76, 82], has been demonstrated to act as a negative allosteric modulator in reducing the effect of 2-AG and 19-THC on CB1 internalization and PLCβ3 and ERK1/2 phosphorylation, exerting a wide range of cellular effects through the endocannabinoid system [83, 84].

The CB2 receptor has long been recognized as an attractive therapeutic target for immune-modulators, pain management, osteoporosis, and the treatment of liver diseases [25-27]. However, compared to CB1, few allosteric modulators are developed for CB2. Pepcan-12 (RVD-hemopressin; RVDPVNFKLLSH), the major peptide of a family of endogenous peptide endocannabinoids (pepcans) found to act as Negative Allosteric Modulators (NAM) of cannabinoid CB1 receptors, has recently been shown to be a potent Positive Allosteric Modulator (PAM) for human CB2 receptor [85]. Martinez-Pinilla *et al*. have provided evidence that CBD at nanomolar concentrations shows the potential not only to slightly but consistently modify the binding of the fluorophore-conjugated CB2-selective compound, CM-157, to HEK-293T cells expressing CB2, but also to significantly reduce the effect of the selective CB2 agonist, JWH133, on forskolin-induced intracellular cAMP levels and on activation of the MAP kinase pathway, suggesting that CBD seems to act as a negative allosteric modulator of the human CB2 receptor [86]. However, more efforts are needed to further characterize small molecules as allosteric modulators of CB2 for future design and synthesis of optimized allosteric modulators.

5. CANNABINOID RECEPTOR BIASED SIGNALING

It has long been believed that most of GPCR couples to multiple Gα proteins (*e.g.* Gαi/o. Gαs, Gαq, or Gα12/13) and β-arrestins, triggering multiple intracellular signalling pathways in parallel and/or sequentially through different transduction mechanisms [87]. Accumulating evidence has firmly established that distinct GPCR agonists exhibit the potential to selectively activate one specific signaling cascade over another, a phenomenon referred to as "biased agonism" or "functional selectivity", and trigger distinct physiological responses [88, 89].

Since for the first time introduction of the pioneering concept of biased agonism in the GPCR field by Kenakin [88], amassing number of GPCR agonists have been identified to preferentially signal *via* either β-arrestin-biased pathway or Gα protein subtype-biased cascade. The first evidence for biased signaling through CB1 came from a study by Glass and Northrup. They demonstrated that agonists HU-210, AEA and WIN55,212-2 evoked maximal Gαi activation, whereas THC induced only partial Gαi activation. In contrast, only HU-210 effected maximal CB1 stimulation of Gαo, with AEA, WIN55,212-2 and THC all partially stimulating [90]. However, agonist-mediated activation of Gαi and Gαo would lead to inhibition of adenyl cyclase. Ligands, HU-210 and CP55,940, were found to differ in potency and efficacy for inhibition of CREB-dependent transcription, leading to opposite effects on AP-1-dependent transcription [91, 92]. Compounds, WIN55,212-2 and HU-210, exhibit the same potential to couple to Gαi and Gαs proteins, whereas both CP55,940 and AEA preferentially activate Gαi-dependent pathway [18, 93]. Based on the structure of indole quinuclidinone (IQD) analogues that act as partial to full CB1 agonists for modulation of Gi-coupled intracellular effector adenylyl cyclase [94], novel agonists PNR-4-20 and PNR-4-02 were developed to act as highly biased Gαi protein agonists with significantly reduced coupling to β-arrestin2 [95]. In contrast, compound ORG27569 has been suggested to selectively activate ERK/12 signaling *via* β-arrestin1 [96, 97]. Accumulating evidence suggests that development of CB1 agonists with biased signaling toward Gαi-dependent pathway, relative to β-arrestin2 signaling, might provide new avenues for the development of analgesic drugs with fewer and less severe adverse effects both acutely and chronically [95-98]. As shown in Fig. (**2**), we summarize the biased signaling at the CB1 receptor.

There are few studies to assess signaling bias at CB2 receptor compared to CB1. Recent investigations have demonstrated that compound CP55,940 exhibits the potential to cause robust internalization of rat CB2, whereas WIN55,212-2 shows no activity in promoting receptor internalization [99]. In contrast, assessment using the Black and Leff operational model indicated that CB2 agonists JWH-133 and THC showed efficacious activity in adenylyl cyclase assays, but failed to associate with β-arrestin [100]. A detailed analysis of biased signaling suggested that THC displayed bias toward ERK1/2 signaling compared to arrestin and GTPγS, when acting at CB2 receptor, while another agonist (R,S)-AM1241 was identified to bias toward arrestin coupling and pERK signaling compared to GIRK channel activation [86].

Cannabinoid receptors, CB1 and CB2, respond to a large amount of synthetic, endogenous and plant-derived cannabinoids [101, 102]. In addition to inhibiting the activity of adenylyl cyclase, CB1 has been shown to be capable of coupling to Gαs [14, 18, 103]. Previous studies have demonstrated that both CB1 and CB2 receptors associate with β-arrestins, resulting in receptor internalization [104, 105], and signal to ERK1/2 signaling pathway *via* either Gβγ or β-arrestin interactions [106]. Additionally, the cannabinoid signaling system has huge potential as a target for therapeutic treatment of many diseases including pain, inflammation,

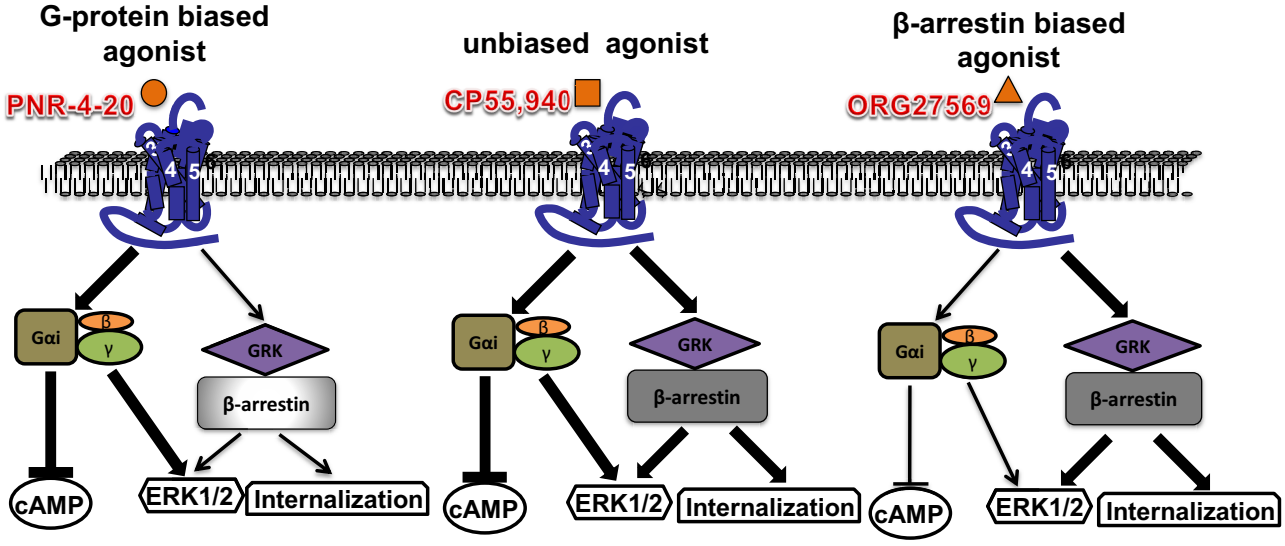

Fig. (2). Schematic representation of biased signaling at CB1. Structurally different ligands will induce diverse conformations of the receptor, which may then favor one of the possible signaling pathways over others. In this diagram, the unbiased agonist CP55,940 engages the CB1 and activates both G protein- and β-arrestin-dependent signaling pathways. PNR-4-20 is biased toward the activation of the Gαi heterotrimer over β-arrestin, while ORG27569 favorably activates β-arrestin.

various mental disorders and so on [107]. Therefore, cannabinoid receptors have been regarded as a particularly ideal model to investigate the biased signaling. However, the investigation of biased agonism for the cannabinoid receptors, CB1 and CB2, is still in its infancy, and more efforts are required for identification and characterization of ligands with biased signaling.

6. SIGNALING OF CANNABINOID RECEPTORS FROM INSIDE CELL

It has been traditionally thought that from their position localized on the cell surface, GPCRs transduce external stimuli into a broad range of cellular responses. However, this has been recently challenged by considerable evidence suggesting that various GPCRs have been found to be functionally localized in the intracellular compartment, including endosomes and trans-Golgi network [108-111], as well as intracellular membranes, including the nuclear membrane [112], endoplasmic reticulum [113], and mitochondrial membrane [114].

CB1 receptor is one of the most abundant G protein-coupled receptors in the brain, and control neuronal activity, metabolism, and functions [115]. Early investigation showed that agonist THC could affect mitochondrial functions [116]. Recent evidence also suggests that CB1 receptor-mediated signaling is found to regulate mitochondrial biogenesis in peripheral non-neural tissues [117, 118]. CB1 receptors were identified to be functionally localized to the outer membrane of neuronal mitochondria (termed ‘‘mtCB1’’), and regulate neuronal energy metabolism [119]. Further characterization demonstrated that upon activation by exogenous cannabinoids and in situ endocannabinoids, mtCB1 induced an intramitochondrial signaling pathway involving G proteins, soluble adenylyl cyclase (sAC), and the protein kinase A (PKA), leading to a decrease of complex I enzymatic activity and respiration in neuronal mitochondria [119, 120]. Mutagenesis analysis pointed to the first 22 amino acids of the CB1 protein responsible for mitochondria localization [120]. A very recent study shows that the CB1 receptor distribution in astrocytes in the mice with genetic restoration of CB1 receptor expression completely matches the endogenous CB1 receptor expression and localization, suggesting the localization of CB1 receptors in astrocyte mitochondria for the first time [121, 122]. However, the physiological role of CB1 receptors in astrocyte mitochondria remains to be elucidated.

In addition to mitochondria, the CB1 receptor has been found to localize to endosomal and lysosomal compartments [123, 124]. The second cannabinoid receptor, CB2, well known to play an important role in the peripheral immune system, was also detected in intracellular localization in prefrontal cortex by subcellular fractionation techniques, western blotting, and binding assay, and agonist stimulation led to inositol triphosphate 3 (IP3) receptor-dependent opening of Ca^{2+}-activated chloride channels and decreased neuronal excitability [123, 125, 126]. Both CB1 and CB2 are likely to exert their functions through intracellular localization. However, more studies are needed to further investigate the molecular mechanisms that determine the subcellular distribution and signaling properties.

CONCLUSION

The endocannabinoid system is an attractive area of research owing to the therapeutic potential for the treatment of pain, obesity, inflammation and a variety of psychiatric disorders. This review provides an overview of current findings regarding the cannabinoid receptor structure, orphan cannabinoid receptors, allosteric modulation, biased signaling, and signaling from intracellularly localized receptors. These findings have changed our fundamental understanding of the endocannabinoid system, from the structural and functional basis for receptor activation to the design and optimization of therapeutic modulators with better efficacy and safety profiles. Further research is required for elucidation of mechanisms involved in biased agonism and signaling of intracellular receptors. Since this manuscript was submitted for publication on the 28th of November 2018, very recent reports on the crystal structure of a signaling cannabinoid receptor 1-G protein complex [127] and crystal structure of the human cannabinoid receptor CB2 were not incorporated [128].

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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