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An Update on Non-CB_1, Non-CB_2 Cannabinoid Related G-Protein-Coupled Receptors

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Abstract

The endocannabinoid system (ECS) has been shown to be of great importance in the regulation of numerous physiological and pathological processes. To date, two Class A G-protein-coupled receptors (GPCRs) have been discovered and validated as the main therapeutic targets of this system: the cannabinoid receptor type 1 (CB_1), which is the most abundant neuromodulatory receptor in the brain, and the cannabinoid receptor type 2 (CB_2), predominantly found in the immune system among other organs and tissues. Endogenous cannabinoid receptor ligands (endocannabinoids) and the enzymes involved in their synthesis, cell uptake, and degradation have also been identified as part of the ECS. However, its complex pharmacology suggests that other GPCRs may also play physiologically relevant roles in this therapeutically promising system. In the last years, GPCRs such as GPR18 and GPR55 have emerged as possible missing members of the cannabinoid family. This categorization still stimulates strong debate due to the lack of pharmacological tools to validate it. Because of their close phylogenetic relationship, the Class A orphan GPCRs, GPR3, GPR6, and GPR12, have also been associated with the cannabinoids. Moreover, certain endo-, phyto-, and synthetic cannabinoid ligands have displayed activity at other well-established GPCRs, including the opioid, adenosine, serotonin, and dopamine receptor families. In addition, the cannabinoid receptors have also been shown to form dimers with other GPCRs triggering cross-talk signaling under specific conditions. In this mini review, we aim to provide insight into the non-CB_1, non-CB_2 cannabinoid-related GPCRs that have been reported thus far. We consider the physiological relevance of these molecular targets in modulating the ECS.

Keywords: cannabinoid receptors; endocannabinoid system; GPCRs; orphan receptors

Introduction

The Class A G-protein-coupled receptors (GPCRs), cannabinoid receptor type 1 (CB_1) and cannabinoid receptor type 2 (CB_2), have been widely confirmed as cannabinoid targets. These receptors have been shown to be involved in numerous physiopathological processes, including pain, inflammation, cancer, metabolic syndromes, hypertension, and neurodegenerative disorders.[1] Nonetheless, the complex pharmacology of the endocannabinoid system (ECS) and its wide implication in numerous biological functions suggest the existence of other receptors playing important physiological roles. Consequently, extensive research is currently focused on the identification of potential missing cannabinoid receptors.

Diverse Class A orphans or lately deorphanized GPCRs have been proposed and evaluated as possible ECS members. Nonetheless, the lack of selective ligands for these receptors along with their intricate signaling pathways is delaying a clear elucidation of their relationship with the ECS. Therefore, thus far no other GPCR has been categorized as the cannabinoid receptor type 3 by the International Union of Pharmacology.[2]

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Herein, we intend to provide an overview of the GPCRs that have been postulated as cannabinoid molecular targets and the current available evidence of their relationship with the ECS. Non-GPCR targets of the cannabinoids such as the peroxisome proliferator-activated receptors, ligand-gated ion channels, or transient receptor potential channels have been revised elsewhere and are beyond the scope of this review.[3,4]

GPR55 and GPR18

Several GPCRs have been postulated to be putative cannabinoid receptors, but so far, only GPR18 and GPR55 have been demonstrated to be targets of a wide variety of endogenous, phytogenic, and synthetic cannabinoid ligands.[4] Despite this fact, inconsistencies in pharmacological data in the literature are hampering their categorization.[5,6]

The cannabinoid-related class A GPCR GPR55 displays low sequence identity with CB_1 and CB_2 (~13% and 14%, respectively). GPR55 is widely expressed in the brain, as well as in the peripheral system, co-localizing with the cannabinoid receptors in diverse tissues.[7–9] This receptor displays G-protein coupling promiscuity associating with $G_{\alpha 13}$,[8,10] $G_{\alpha q/11}$,[11] $G_{\alpha 12,}$[11] or $G_{\alpha 12/13}$[8,12] depending on the cell line or tissue. GPR55 has been implicated in different physiopathological conditions such as cancer,[13–15] pain,[11,16,17] metabolic disorders,[18,19] vascular functions,[20,21] bone physiology,[22] and motor coordination.[23]

The phospholipid lysophosphatidylinositol (LPI) is considered the endogenous GPR55 ligand.[8,24,25] In fact, GPR55 has also been named the LPI1 receptor.[26] Numerous CB_1 and CB_2 ligands have also been reported to act as GPR55 modulators.[6,27–29] However, significant pharmacological discrepancies have been found depending on the tested functional outcome.[6] For instance, the well-known phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) displayed activation of GPR55 according to certain reports,[10,11] while it was unable to exert any effect in other functional assays.[24,30] Cannabinoid ligands reported to be recognized by GPR55 and their intriguing pharmacology have been recently reviewed elsewhere.[31]

Although its sequence presents low identity with CB_1 and CB_2 (~13% and 8%), GPR18 has also been tightly associated with the ECS.[4,32] High expression of GPR18 has been found in the lymphoid tissues, while it is moderately expressed in other organs such as lungs, brain, testis, or ovary.[33,34] Initially, GPR18 was found to couple to $G_{\alpha i/o}$; however, subsequent findings suggested the participation of the $G_{\alpha q/11}$ transduction pathway as well.[34–36] Different reports have shown the therapeutic potential of this target in the treatment of pathologies such as intraocular pressure,[37] cancer,[38] or metabolic disorders[39] among others.

N-arachidonoyl glycine (NAGly) has been suggested to be the endogenous GPR18 ligand by several research groups.[32,34] However, other researchers were not able to confirm these data.[40] Recent investigations point to the existence of another endogenous GPR18 activator: the polyunsaturated fatty acid metabolite, Resolvin D2 (RvD2), which is mainly involved in inflammatory processes.[41] In addition, and despite the pharmacological divergences observed among some reports, GPR18 has been shown to recognize an array of CB_1 and/or CB_2 ligands of endogenous, phytogenic, or synthetic nature (reviewed by others).[39,42]

The pharmacological discrepancies on the appraisal of cannabinoids in these two receptors, as well as the lack of selective ligands targeting them, are delaying an insightful understanding of the relation of GPR55 and GPR18 with the ECS. These inconsistencies, which may rely on intrinsic properties of these GPCRs, or on the cell type or functional assay, need to be further studied. Intensive efforts are also focused on the structural understanding of these receptors,[43] as well as the development of more potent and selective pharmacological tools for the study of these promising therapeutic targets.

GPR3, GPR6, and GPR12

GPR3, GPR6, and GPR12 are three orphan Class A GPCRs that exhibit a very close phylogenetic relationship with the cannabinoid receptors CB_1 and CB_2 (Fig. 1). Indeed, they belong to the same cluster of receptors, the so-called MECA cluster (which consists of the melanocortin receptors, the endothelial differentiation GPCRs, the cannabinoid receptors, the adenosine binding receptors, and the orphan receptor subset GPR3, –6, and –12).[44,45] Because of their phylogenetic proximity, these orphan receptors share common conserved residues and unique sequence motifs with CB_1 and CB_2.[46] According to Fredriksson et al. these orphan receptors may share a common ancestor with the cannabinoid receptors since they share the same chromosomal positions.[45]

GPR3, GPR6, and GPR12, which share over 60% of sequence similarity, were first cloned in 1995.[47,48] These receptors constitutively activate adenylate cyclase by coupling to $G_{\alpha s}$ proteins. In fact, different

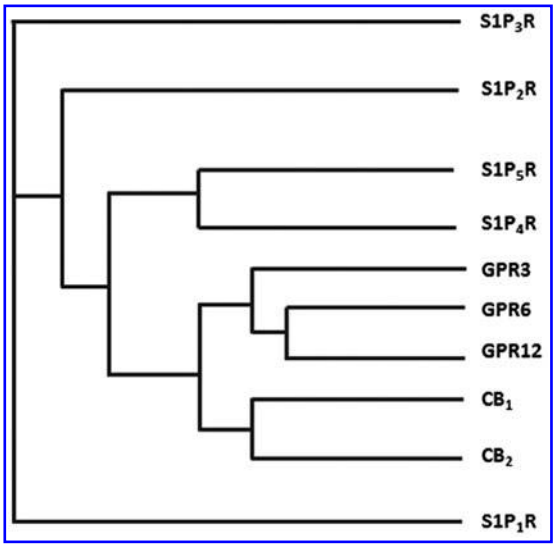


FIG. 1. Phylogenetic tree of cannabinoid receptors and the closely related Class A GPCRs (S1PR family and the orphan receptors GPR3, GPR6, and GPR12). Data were obtained from GPCRdb.org. CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; GPCR, G-protein-coupled receptor; S1P, sphingosine-1-phosphate.

groups have reported that when expressed in diverse cell lines, they can stimulate adenylate cyclase to levels similar in amplitude to agonist-activated GPCRs.[47,49,50] In addition to $G_{\alpha s}$, GPR6 and GPR12 have also been suggested to couple to $G_{\alpha i/o}$,[51,52] but further investigations are required to confirm this G-protein promiscuity.

GPR3, GPR6, and GPR12 are predominantly expressed in the brain and the reproductive system.[49] This family of constitutively active GPCRs is involved in neuronal differentiation and growth, as well as in the formation of synaptic contacts.[49] Therefore, their role in different neurological processes such as neurite outgrowth,[49] Alzheimer's disease,[53–57] development of cerebellar granule neurons,[58,59] neuropathic pain,[60] early phases of cocaine reinforcement,[61] emotional-like responses,[62] instrumental learning,[63] or Parkinson's disease[64,65] has been studied. Other pathophysiological conditions such as oocyte maturation,[66,67] dyslipidemia,[68] and cell proliferation[69] may also be impacted by the modulation of some of these receptors.

The bioactive lipids, sphingosine-1-phosphate[50,52] and/or sphingosylphosphorylcholine,[51] have been proposed as endogenous ligands of these receptors (Fig. 2). However, other groups were not able to confirm this claim, and consequently, GPR3, GPR6, and GPR12 are still categorized as orphans.[30,70,71] Interestingly, among the very few ligands discovered so far for these receptors, the nonpsychoactive phytocannabinoid cannabidiol (CBD) stands out as being able to target GPR3 and GPR6,[72] acting as a β-arrestin2 inverse agonist of both receptors. This functionality is of high interest in the GPR3 field because β-arrestin2 signaling at GPR3 has been directly linked to the manufacture of beta-amyloid plaque ($A\beta_{1-40}$ and $A\beta_{1-42}$) in Alzheimer's disease through complex formation with γ-secretase.[56,57] Because CBD is an inverse agonist of this signaling pathway at GPR3, it may represent a potential tool for the reduction of amyloid pathology. Other phytocannabinoids and several endocannabinoids were also tested but so far none of them were found to modulate this family of orphan receptors.[30,72]

So, a relationship between the cannabinoids and the orphan receptors GPR3, GPR6, and GPR12 has been evidenced. Nonetheless, extensive research and more pharmacological tools are needed to extract significant conclusions about the association of these receptors with the ECS and its ligands.

Alkylindole-Sensitive Receptors

As reported by different research groups, the well-known aminoalkylindole cannabinoid agonist WIN55,212-2 (Fig. 3) displays pharmacological functions independent of the cannabinoid receptors CB_1 and CB_2.[73–75] This fact led to the identification of novel targets commonly referred to as the alkylindole (AI)-sensitive receptors.[74,76,77] These cannabinoid-related receptors are modulated by AI derivatives, but not by other classes of cannabinoid ligands.[76] Diverse evidence suggests that the AI-sensitive receptors are $G_{\alpha s}$-protein coupled receptors that are mainly expressed in microglia and astrocytomas.[76–79] However, their biological functions, pharmacology, and therapeutic value remain to be unraveled due to the lack of selective pharmacological tools.

Recent studies from Stella and coworkers revealed the role of AI-sensitive receptors in the modulation of microglial cell migration and proliferation highlighting their potential in the treatment of gliomas.[77,78] Moreover, these authors have identified a series of naphthoyl AI derivatives, ST-11, ST-23, ST-25, and ST-48 (Fig. 3) among them, that bind to the AI-sensitive receptors.[78] These compounds display affinities in the nanomolar range when competing with [^{3}H]WIN55,212-2 in DBT

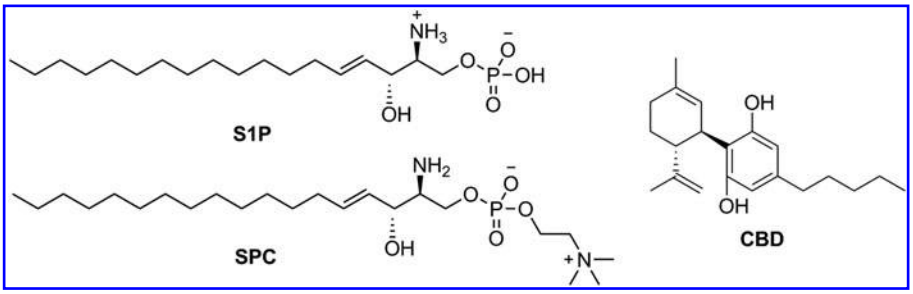


FIG. 2. Structures of the putative GPR3, GPR6, and GPR12 endogenous ligands S1P and SPC and the GPR3 and GPR6 inverse agonist CBD. SPC, sphingosylphosphorylcholine.

(Delayed Brain Tumor) cells which endogenously express AI-sensitive receptors, while lacking CB_1 and CB_2 receptors.[80] Compound ST-11 stands out from this study because of its potency at AI-sensitive receptors, while not interacting with CB_1 and CB_2 receptors. In addition, *in vitro* assays revealed that this compound inhibits cell migration and proliferation in the aforementioned mouse glioma cell line, DBT. Further studies revealed that ST-11 can reduce glioblastoma growth in a syngeneic mouse model.[81]

Even though extensive research is clearly needed to understand the pathophysiological function of these receptors, reported data suggest that AI-sensitive receptor agonists could represent a novel class of potential brain cancer antitumor drugs.

Cannabinoid-Related Oligomers

Numerous studies have shown that GPCRs, cannabinoid receptors among them, can exist and function as dimers or complexes of higher order.[82–85] This oligomerization may affect receptor signaling, receptor trafficking, and ligand binding. The physiological relevance of such dimerization has not yet been fully established for the cannabinoid receptors; nonetheless, the presence of cannabinoid homo- and heterodimers in specific tissues has been intensely reported over the last years.

For the CB_1 receptor, heteromers have been suggested to exist under certain physiological conditions with serotonin,[86] angiotensin,[87] opioid,[88–90] GPR55,[91] somatostatin,[92] orexin,[93,94] dopamine,[95–97] and adenosine[98] receptors among others (Table 1). Although CB_2 has been less investigated, recent research revealed that it may form heterodimers with CB_1,[99] with GPR55,[100,101] with the serotonin receptor $5HT_{1A}$,[102] or with the chemokine receptor CXCR4.[103] The expression of these heterodimers has been associated with different pathologies. For instance, the CB_2−CXCR4 and the CB_2−GPR55 dimers have been associated with cancer progression, while the CB_1−A_{2A} and the CB_1−D2 heteromers have been suggested to have physiological implications in neurodegenerative disorders such as

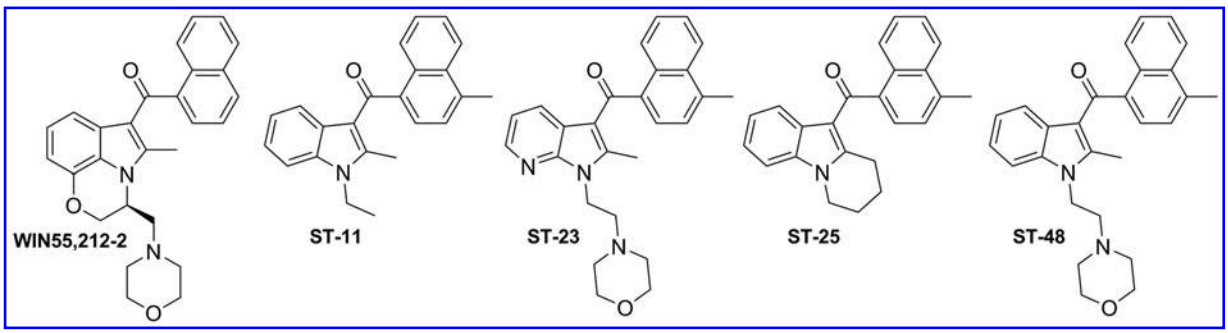
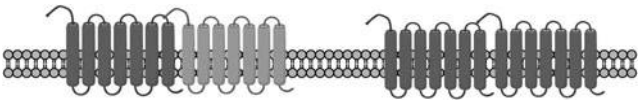


FIG. 3. Alkylindole derivatives WIN55,212–2, ST-11, ST-23, ST-25, and ST-48.

Table 1. Cannabinoid-Related G-Protein-Coupled Receptor Dimers Reported So Far



Heterodimers		Homodimers	
CB_1—D2	95,97	CB_1—CB_1	104–106
CB_1—A_{2A}	98	CB_2—CB_2	107–109
CB_1—$5HT_{2A}$	86		
CB_1—AT_1	87		
CB_1—GPR55	91		
CB_1—SST5	92		
CB_1—OX1	94		
CB_1—OX2	93		
CB_1—μOR	90		
CB_1—δOR	88		
CB_1—CB_2	99		
CB_2—GPR55	100,101		
CB_2—$5HT_{1A}$	102		
CB_2—CXCR4	103		

CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2.

Huntington's or Parkinson's diseases. All these data suggest that the ECS interacts in a significant manner with several other endogenous systems.

With regard to cannabinoid receptor homodimerization, more data have been published on CB_1 homodimers than on their CB_2 counterparts. The presence of CB_1 receptor homodimers has been reported in different biological tissues,[104–106] but their functional role has not been determined. In contrast, CB_2 homodimers have been evidenced,[107–109] but their pharmacological potential has not been explored yet.

In this field, bivalent ligands have emerged as promising new pharmacological entities and potential tools for the biological study of their respective dimeric receptors.[110–113] Despite their poor pharmacokinetic properties,[114] bivalent ligands can exhibit enhanced activity and selectivity over their respective corresponding parent ligands offering unique pharmacological strategies. Bivalent ligands have been synthesized and evaluated for several GPCRs. Opioid,[115,116] dopamine,[117,118] and histamine[119] are some of the receptors for which a bivalent compound provided higher activity than their monomer counterparts. CB_1 homobivalent[120–122] and heterobivalent[123–125] ligands have been also reported and explored. However, currently available receptor structural information challenges the fact that bivalent ligands can simultaneously bind to both receptors within the dimer, especially in the case of lipid receptors as the cannabinoids.[126] Therefore, novel drug design approaches to target dimers, as well as new techniques to determine bivalent binding, remain to be explored.

Homo- and heterodimerization likely influences the manner in which the ECS responds to ligands. Nevertheless, unambiguous data about their physical association in native tissues, as well as their pharmacology, are needed to clearly identify what biological functions are impacted by cannabinoid dimers.

Well-Established GPCRs Related to the Cannabinoids

Certain endo-, phyto-, and synthetic cannabinoid ligands have been shown to modulate well-known GPCRs. These GPCRs include members of established families such as the opioid, serotonin, muscarinic, dopamine, and adenosine families. For instance, the endocannabinoid anandamide has been shown to act at the adenosine receptor A_3,[127] the muscarinic acetylcholine receptors M1 and M4,[128] and the serotonin receptors 5-HT_{1A} and 5-HT_{2A}[129] among others. In addition, phytocannabinoids such as Δ^9-THC and CBD have been shown to modulate the μ— and δ—opioid receptors,[130] while other plant-derived compounds such as CBG (cannabigerol) and Δ^9-THCV (tetrahydrocannabivarin) display activity at the 5-HT_{1A} receptor.[131,132] Likewise, synthetic cannabinoids, such as the CB_1 inverse agonists taranabant (MK-0364) and rimonabant (SR141716), have also displayed activity in well-established targets. These include the adenosine A_3 and the tachykinin NK2 receptors.[133]

Some of these cannabinoid ligands have been proposed to interact allosterically with the aforementioned targets. It is worth mentioning that the efficacy that most of these cannabinoids exhibit toward these GPCRs is lower than the one displayed at the CB_1 and/or CB_2 receptors. Therefore, there is no evidence indicating a necessary recategorization of these receptors.

Other GPCRs

Because of their ability to recognize lipids and their relatively close phylogenetic relationship with CB_1 and CB_2, several other Class A orphan or recently deorphanized GPCRs such as GPR40, GPR43, GPR41, GPR120 (currently classified as free fatty acid receptors FFA1, FFA2, FFA3, and FFA4, respectively), GPR23, GPR92 (recently categorized as lysophosphatidic acid receptors LPA4 and LPA5), GPR84, GPR119, or GPR35 have been postulated as possible cannabinoid receptor candidates.[4] However, there is no available evidence since they do not meet some of the criteria established by the International Union of Pharmacology.[4,70]

Conclusions

Two cannabinoid receptors, CB_1 and CB_2, have been validated at the molecular level as the main targets of the ECS. These two GPCRs have been widely explored in the development of numerous pathophysiological processes, and their therapeutic potential for the treatment of different diseases has been extensively confirmed.[1] Great efforts are being made to structurally understand these receptors; in fact, CB_1 in its inactive[134,135] and active[136] states has been recently crystallized. Despite possible crystallization artifacts, these structures will help shedding light into the complex pharmacology of the cannabinoid receptors.

Growing evidence suggests that other cannabinoid or cannabinoid-like receptors remain to be identified as important players of the ECS. Different endogenous, phytogenic, and/or synthetic cannabinoid ligands have been reported to modulate GPCRs such as GPR18, GPR55, GPR3, GPR6, or the AI-sensitive receptors, among others. Pharmacological discrepancies and the lack of selective ligands for these receptors are delaying the characterization of their relationship with the ECS. Consequently, no CB_3 receptor has yet been confirmed.[2]

Adding more complexity to the ECS scenario, molecular interactions of the cannabinoid receptors with other GPCRs have been reported. Co-localization or co-immunoprecipitation data suggest the presence of cannabinoid homo- and heterodimers in specific native tissues. Cannabinoid receptor dimerization may not only influence the pharmacology of these receptors but also it may provide new signaling pathways through the interacting protomers. However, due to the lack of appropriate tools, there is still limited *in vivo* information about the expression of cannabinoid dimers. Hence, it remains a challenge to elucidate their therapeutic relevance under specific physiological conditions.

Currently, appropriate characterization of cannabinoid ligands should take into account the activity at the aforementioned GPCRs. Possible biased agonism of ligands, allosterism, or cross-talk signaling could be determining the intricate GPCR pharmacology. In addition, differential coupling and regulation of G-proteins or the formation of oligomers are among GPCR intrinsic properties that might be delaying the validation of novel potential cannabinoid targets. Therefore, further research is needed to fully understand the physiopathological role of these non-CB_1, non-CB_2 GPCRs in the modulation of the ECS.

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Author Disclosure Statement

No competing financial interests exist.

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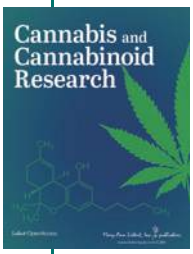
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Abbreviations Used

AI = alkylindole
CB_1 = cannabinoid receptor type 1
CB_2 = cannabinoid receptor type 2
CBD = cannabidiol
ECS = endocannabinoid system
GPCR = G-protein-coupled receptor
LPI = lysophosphatidylinositol
THC = tetrahydrocannabinol

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