Published in final edited form as:
Pharmacol Ther. 2009 May ; 122(2): 83–96. doi:10.1016/j.pharmthera.2009.01.005.

The therapeutic potential of novel cannabinoid receptors

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Abstract

Cannabinoids produce a plethora of biological effects, including the modulation of neuronal activity through the activation of CB_1 receptors and of immune responses through the activation of CB_2 receptors. The selective targeting of either of these two receptor subtypes has clear therapeutic value. Recent evidence indicates that some of the cannabinomimetic effects previously thought to be produced through CB_1 and/or CB_2 receptors, be they on neuronal activity, on the vasculature tone or immune responses, still persist despite the pharmacological blockade or genetic ablation of CB_1 and/or CB_2 receptors. This suggests that additional cannabinoid and cannabinoid-like receptors exist. Here we will review this evidence in the context of their therapeutic value and discuss their true belonging to the endocannabinoid signaling system.

Keywords

cannabinoid; CB_1; CB_2; non-CB_1/CB_2

I. Introduction

For centuries, the plant *Cannabis sativa (C. sativa)*, commonly known as marijuana, has been used for a variety of recreational, religious, and medicinal purposes across diverse cultures. The first recorded medicinal attributes of *C. sativa* in western medicine were its powerful sedative, anticonvulsant, and analgesic properties (Mechoulam 1986). Furthermore, it was one of the most commonly prescribed medicines in the U.S. pharmacopoeia until its criminalization in the late 1930s (Belenko 2000), resulting in a near standstill of scientific research for the next 30 years. The discovery and identification of Δ^9-tetrahydrocannabinol (Δ^9 -THC) as the primary bioactive constituent in *C. sativa* revived the interest of the scientific community to reconsider the therapeutic potential of such compounds. The subsequent design of synthetic and radiolabeled compounds, and the use of molecular biology to identify their targets led to the discovery of the two cannabinoid receptors (CBRs) that belong to the endocannabinoid signaling system (eCBSS), enabling researchers to better investigate the medicinal properties of cannabinoids at the molecular level.

The eCBSS is involved in basic physiological processes throughout the central nervous system (CNS) and in the periphery, regulating a multitude of cognitive, homeostatic, and

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immunological functions. Targeting specific components of the eCBSS may be of therapeutic value for cancer cachexia, victims of acute and chronic pain, neurological disease, and autoimmune disorders such as multiple sclerosis. However, some of the more recently identified components belonging to the eCBSS have resisted molecular identification, complicating the development of selective cannabinoid-based therapy. These novel CBRs, which are responsible for some of the observed non-CBR mediated effects in the periphery and CNS, are currently under intense investigation. Before we discuss the studies that have led to the pharmacological identification of these novel receptors, we will first provide an overview of what is currently known about the CBRs and other eCBSS components.

II. Cannabinoid compounds, receptors, and the endocannabinoid signaling system

The medicinal and euphoric properties ascribed to *C. sativa* are principally due to phytocannabinoids, a family of bioactive constituents produced by this plant. In 1965, Gaoni and Mechoulam described the isolation and chemical nature of Δ^9 –THC, the primary psychoactive phytocannabinoid of *C. sativa* (Mechoulam & Gaoni 1967) (Figure 1). Based on the lipophilicity of Δ^9 –THC, it was initially hypothesized that it might mediate its biological effects by disrupting cellular membrane fluidity and phytocannabinoids were thus classified as “partial anesthetics.” However this concept was rapidly challenged and ultimately invalidated by the classic structure-activity analyses of Δ^9 –THC’s ability to inhibit adenylyl cyclase activity through $G_{i/o}$-proteins, clearly indicating a receptor-mediated mechanism (Dill & Howlett 1988). This landmark discovery and the subsequent synthesis of additional cannabinoid compounds led to the molecular identification of two G-protein coupled receptors (GPCRs): the cannabinoid 1 (CB_1) (Devane *et al.* 1988; Matsuda et al. 1990) and cannabinoid 2 (CB_2) receptors (Munro et al. 1993).

CB_1 receptors are predominantly expressed by neurons, while CB_2 receptors are predominantly expressed by immune cells (Munro et al. 1993), a dichotomy that has outstanding therapeutic potential. To date over 60 phytocannabinoids have been identified (Dewey 1986), some behaving as agonists or antagonists with varying affinities for CB_1 and/or CB_2 receptors, and a large portion of their cannabimimetic effects are mediated through these two GPCRs. Both receptors are seven-transmembrane proteins that couple to guanine-nucleotide-binding proteins (G- proteins) and inhibit adenylyl cyclase activity through the α subunit of the G-protein-signaling complex (Dill & Howlett 1988; Matsuda et al. 1990) and activate ERK through the βγ subunit of this complex (Bouaboula et al. 1995; Shoemaker et al. 2005). CB_1 receptors modulate synaptic transmission by inhibiting calcium channels and possibly activating potassium channels on presynaptic terminals (Gebremedhin et al. 1999; Mackie & Hille 1992; Mackie et al. 1995; McAllister et al. 1999). CB_2 receptors regulate immune responses by regulating immune cell migration, cytokine production, and antigen presentation (for review see Miller & Stella 2008). It should be noted that CB_1 and CB_2 receptors are also expressed in many other cell types in the brain and peripheral tissue, however their role in these tissues are only starting to be understood. For instance, in vitro evidence suggest that both CB_1 and CB_2 receptors are expressed by astrocytes and may participate in regulating neuroinflammation and provide neuroprotection by tempering lipopolysaccharide (LPS)- and IL-1β-induced NO synthesis, as well as inhibiting the production of other inflammatory mediators (Molina-Holgado et al. 2002; Sheng et al. 2005). A more recent publication demonstrates the involvement of astrocytic CB_1 receptors mediating the communication of eCBs between neurons and astrocytes (Navarrete et al. 2008). CB_2 receptors are also expressed by a small population of brainstem neurons (Van Sickle et al. 2005), and by other selective tissue populations (Ross et al. 2001; Stander et al.

2005; Wotherspoon et al. 2005), however the role of this cannabinoid receptor subtype in these cells is only starting to be understood.

Although the eCBSS has been extensively studied, many basic questions remain unanswered. For instance, pharmacological studies revealed several non-CB_1/CB_2-mediated events (Begg et al. 2005), which suggest two possibilities: cannabinoids may produce receptor-independent effects (Howlett & Mukhopadhyay 2000; Maingret et al. 2001; Oz 2006) and/or receptor-dependent effects through receptors distinct from CB_1 and CB_2. While the former possibility constitutes an interesting prospect, we have chosen to focus this review on the evidence for novel receptors, specifically GPCRs, engaged by cannabinoid compounds.

III. Pharmacological identification of novel cannabinoid and cannabinoid-like receptors using mice lacking CB_1 and CB_2 receptors

The pharmacology of cannabinoid compounds is rich, consisting of a vast array of CB_1 and CB_2 specific agonists and antagonists. Currently, there are five classes of cannabinoid ligands. The first class include all the classical cannabinoids, which are tricyclic-dibenzopyran derivatives isolated from the plant *C. sativa* (including Δ^9-THC) or close synthetic analogues such as HU-210 (Figure 1). These compounds bind non-selectively to CB_1 and CB_2 receptors (Table 1). The second class of compounds consists of non-classical cannabinoids, which are structurally similar to the classical cannabinoids, but are AC-bicyclic and ACD-tricyclic analogues lacking the dihydropyran ring (Figure 1). The prototype of compound belonging to this class is CP55940, a full agonist at both the CB_1 and CB_2 receptors (Table 1). Aminoalkylindoles make up the third class of compounds, the prototypical compound being WIN55,212-2, a full agonist at both CBRs, that exhibits an approximately two fold higher affinity toward CB_2 over CB_1 (Felder et al. 1995). Aminoalkylindoles are structurally dissimilar from both the classical and non-classical cannabinoid compounds (Table 1 and Figure 1). The fourth class of cannabinoid ligands encompasses arachidonic acid derivatives. These endogenous ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), behave as partial and full agonists at CB_1 and CB_2, respectively (Table 1). The fifth class of cannabinoids consists of the diarylpyrazole compounds, including SR141716A and AM251 (inverse agonists at CB_1 receptors) (Felder et al. 1995; Rinaldi-Carmona et al. 1994; Ruiu et al. 2003).

In summary for this section, although some of the aforementioned drugs were originally designed to selectively target CB_1 and/or CB_2 receptors, it has now become evident that the selectivity of some of these compounds is questionable. As we will see below, recent studies suggest that cannabinoids bind and activate at least three additional receptors. Since GPCRs constitute the most widely targeted proteins to modify physiological functions and pathological processes, the development of pharmacological agents that selectively interact with such novel receptors (especially if they are devoid of the unwanted side effects associated with cannabinoids acting at CB_1 receptors) opens the prospect for entirely novel therapeutic venues. The evidence that these novel receptors are involved in vasodilation, neuroinflammatory pain, and synaptic transmission (see Table 3) is described in the following sections.

a.) Novel cannabinoid receptors expressed by endothelial cells

Cardiovascular disease is the leading cause of death in the U.S. and over 80 million adults currently suffer from this devastating illness as quoted by the American Heart Association. With a growing body of evidence supporting the therapeutic effects of cannabinoids acting on the vasculature, targeting the eCBSS clearly constitutes a promising option for the next

generation of cardiovascular therapeutics (Pacher et al. 2005; Pacher et al. 2008; Randall et al. 2002). Within this context, there is convincing and very exciting evidence for a novel receptor engaged by cannabinoids to regulate vasodilation (Kunos et al. 2002).

The initial non-CB_1/CB_2 mediated cannabinoid effect shown to modulate the vasculature was ascribed to the "AEA endothelial receptor" (AeR) because of its sensitivity to AEA (Jarai et al. 1999; Offertaler et al. 2003; Wagner et al. 1999). Subsequent studies identified additional compounds targeting this as-yet-uncloned receptor, which helped advance its pharmacological characterization. The phytocannabinoid cannabidiol (CBD) is an important bioactive component of *C. sativa* that has outstanding therapeutic potential, for it does not produce psychotropic effects and has been shown to act as an inverse agonist at both CB_1 and CB_2 receptors (Thomas et al. 2007). More specifically, CBD interacts with CB_1 receptors in an allosteric manner (Thomas et al. 2007). Allosteric modulation of GPCR constitutes an exciting new field of research and compounds that modulate CB_1 receptors by this mechanism hold tremendous therapeutic value (Price et al. 2005). Evidence suggests that CBD may also interact with novel cannabinoid receptors, although the details of this interaction are still unknown. While an initial report showed that CBD antagonize the novel AeR-mediated vasodilation (Jarai et al. 1999), several follow-up reports showed that the CBD analogue O-1918 and the synthetic isomer of CBD, abnormal-cannabidiol (abn-CBD), antagonize and activate the AeR (Ho & Hiley 2003; Mo et al. 2004; Offertaler et al. 2003; Wagner et al. 1999). More specifically, the latter compound causes vasodilation of mesenteric arteries in mice lacking CB_1 and CB_2 receptors (Jarai et al. 1999), a reason for some laboratories, including ours, to refer to this receptor as the "abn-CBD receptor" (Walter et al. 2003). Thus, the pharmacological profile of the AeR does not parallel the pharmacology of CB_1 and CB_2 receptors since WIN55,212-2, Δ^9-THC, and HU-210 lack efficacy at this target (Begg et al. 2003; McCollum et al. 2007; Wagner et al. 1999). Based on these findings, it is now generally accepted that most of the cannabinoid-induced vasodilation is mediated through this distinct target, and in line with this notion, the CB_2 antagonists SR144528, AM281 and AM630, and the CB_1 antagonist AM251 do not affect the AeR-mediated vasodilatory effect (Herradon et al. 2007; Ho & Hiley 2003).

The pharmacological tools shown to target AeR have greatly helped characterize some of the molecular and cellular mechanisms involved in the non-CB_1/ -CB_2 mediated modulation of vasodilation. For example, compounds acting through AeR signals in an endothelial-dependent manner because endothelial denudation of the tissue will abolish cannabinoid-induced vasodilation (Ho & Hiley 2003; Wagner et al. 1999). The mechanisms involved in the abn-CBD induced vasodilation might depend on the activation of potassium channels in rat mesenteric arteries that appear to be calcium-sensitive in human umbilical vein endothelial cells (HUVEC) (Begg et al. 2003; Ho & Hiley 2003). In rabbit aortic endothelial cells (RAEC) the PI3K/Akt pathway is activated by methanandamide (mAEA), a metabolically stable analog of AEA. Activation of PI3K/Akt in endothelial cells by mAEA (EC_{50} = 9.4 nM) results in the phosphorylation of eNOS and subsequent increased NO synthesis, a mechanism likely initiating the vasodilation (McCollum et al. 2007). Accordingly, pharmacological inhibition of PI3K with LY294002 and of $G_{i/o}$ proteins with PTX decreases the synthesis of NO mediated by mAEA (McCollum et al. 2007). The biological effect of abn-CBD in HUVEC also involves the activation of PI3K/Akt in a $G_{i/o}$-protein dependent manner (Offertaler et al. 2003). The dual activation of p42/44 MAP kinase in HUVEC was also observed following abn-CBD treatment, a result not reported in RAEC (Offertaler et al. 2003). Thus, while at first glance the pharmacological profile of AeR and signaling pathways coupled to this receptor may appear consistent across most studies, some interesting inconsistencies also exist. McCollum *et al.* (2007) reported that mAEA induces vasodilation of RAECs in a SR141716A-insensitive manner whereas Wagner et al. (1999) reported that both the AEA- and mAEA-mediated regulation of

vasodilation of rat mesenteric arteries was SR141716A-sensitive (Wagner 1999). A parsimonious explanation for these pharmacological inconsistencies could be that differences in tissue preparations might lead to different pharmacological and cellular responses. Alternatively, and in our opinion more thought-provoking, these discrepancies may reflect the following two possibilities: 1) the presence of yet another receptor in the vasculature different from the AeR or 2) that the AeR is subject to "agonist-induced trafficking" and resulting differential modulation of effector signaling. Be what it may, these two sets of evidence support the existence of non-CB_1/CB_2 receptors that carry tremendous promise for novel therapies aimed to treat and combat cardiovascular disease.

Another player that might also engage AeR is oleamide, an endogenous lipid structurally related to AEA. This ligand exhibits cannabinomimetic effects despite its lack of affinity at either CB_1 or CB_2 receptors, although its relevance remains controversial (Fowler 2004; Leggett et al. 2004). In favor of its action at AeR, oleamide causes vasodilation in rat mesenteric arteries that is partially endothelium-dependent. Transient receptor potential (TRP) channels and potassium-sensitive calcium channels are involved in this oleamide-mediated effect, even though this effect is blocked by SR141716A and O-1918, and not by AM251 (Hoi & Hiley 2006). This effect of oleamide on the vascular tone is PTX-sensitive, suggesting a $G_{i/o}$-mediated mechanism (Hoi & Hiley 2006). In a similar study, the novel water soluble cannabinoid-like agonist VSN16 also causes vasodilation in rat mesenteric arteries, an effect that is sensitive to O-1918 and SR141716A, and involves TRP channel activation (Hoi et al. 2007). However, in this case, pre-treatment with PTX did not affect the VSN16 elicited vasodilation (Hoi et al. 2007). We noted that the same tissue was used in both studies, suggesting differential effector coupling induced by these drugs, possibly through PTX-sensitive and PTX-insensitive mechanisms.

An additional study investigated the role of yet another endogenous lipid, *N*-arachidonoyl-L-serine (ARA-S), also structurally related to AEA. Although ARA-S binds with minimal affinity to CB_1, CB_2, and TRPV1, it produces endothelium-dependent vasodilation in rat isolated mesenteric arteries (K_i = 550nM) and abdominal aorta (K_i = 1.2 μM) (Milman et al. 2006). The effects of ARA-S are PTX-sensitive in the abdominal aorta and in cultured HUVEC, congruent with other studies suggesting that cannabinoid-induced vasodilatory effects are mediated by $G_{i/o}$ (Milman et al. 2006). However, it should be emphasized that ARA-S also possesses PTX-insensitive effects: ARA-S inhibits lipopolysaccharide (LPS) induced TNFα production in mesenteric arteries in a PTX-insensitive manner, a response inhibited by O-1918 (Milman et al. 2006). Because O-1918 can inhibit this effect, one can postulate that both ligands bind to one target and that the PTX-insensitive component is a unique feature of ARA-S, a result that agrees with differential agonist-induced trafficking at the AeR, similarly to what has been elegantly shown for CB_1 and CB_2 receptors (Mukhopadhyay et al. 2002; Shoemaker et al. 2005). In other words, this evidence points toward an endothelial receptor that couples differentially to signal transduction pathways in a ligand-dependent manner. The integration of these pathways with those coupled to CB_1 receptors may be responsible for the complex cannabinoid-mediated vasodilatory effects.

In summary, it is clear that a novel endothelial CBR mediating the response induced by specific cannabinoid compounds exists. While the reported pharmacological profile remain to be thoroughly understood, it is likely that this receptor couples to distinct effectors in a ligand-dependent manner and thus is able to differentially control vasodilation. However before a specific therapeutic outcome may be unequivocally linked to the targeting of this receptor, it will be necessary to determine its molecular identity. The subsequent pharmacological verification of the cannabinoid-mediated vasodilatory effects in genetically modified animal models lacking the AeR will allow for a more thorough understanding of the molecular mechanisms involved in cannabinoid-mediated regulation of vascular tone.

b.) Novel cannabinoid receptors modulating analgesia

More than 50 million Americans suffer from some form of chronic pain as reported by the NIH pain and research programs. Many cases of chronic pain cannot be relieved by current therapies, highlighting the need for alternative strategies to treat these patients. Furthermore, many analgesics, including opioids and non-steroidal anti-inflammatory drugs (NSAIDs), cause significant side effects associated to their long term use. Because cannnabinoids induce analgesia in both acute and chronic pain models (Guindon & Hohmann 2008), their therapeutic potential as analgesics is being evaluated.

While it is clear that activation of CB_1 and CB_2 receptors induces analgesia, several evidence show that some of the cannabinoid-mediated analgesic responses are not mediated through these two receptor subtypes. A remarkable such example involves a particular endogenous acylethanolamide, palmitoylethanolamide (PEA), which does not activate either CB_1 or CB_2 receptors (Showalter et al. 1996), and yet its analgesic property is sensitive to the CB_2 antagonist SR144528 (LoVerme et al. 2005). This may be interpreted in two different ways: PEA and SR144528 bind to a single receptor target, suggesting that SR144528 is non-specific, or PEA binds to a site distinct from SR144528 binding and their subsequent signaling may converge. In either case, PEA is likely acting through a novel receptor target site. This analgesic effect of PEA was first recognized when local administrations of both AEA and PEA were shown to induce classic analgesic paradigms (Calignano et al. 1998). Specifically, AEA inhibits the early phase of formalin-induced pain in a localized manner that is mediated by CB_1 receptors expressed on the peripheral nerve endings of sensory neurons (Calignano et al. 1998; Jaggar et al. 1998), whereas PEA inhibits both the early and late phases of formalin-induced pain independently of CB_1, CB_2 and μ-opioid receptors (Calignano et al. 1998). Thus, while the authors convincingly concluded that AEA and PEA act synergistically when co-administered to counteract formalin-induced nociception, the molecular details of PEA's action remained unknown. This synergistic effect should be considered in light of AEA and PEA being co-released under certain pathophysiological conditions (Di Marzo et al. 1994), which would lead to more robust and relevant analgesia compared to the mere isolated release of AEA (LoVerme et al. 2005; Maccarrone et al. 2002a; Maccarrone et al. 2002b; Mechoulam et al. 1998).

What is the molecular target of this analgesic effect induced by PEA? Both AEA and PEA interact with peroxisome proliferator activated receptor-α (PPAR-α), and thus this subtype of receptor might be involved in part of their actions as analgesics (Lo Verme et al. 2005; Sun et al. 2007). These receptors belong to the nuclear receptor superfamily and are often linked to lipid metabolism and inflammation (reviewed in Burstein 2005; O'Sullivan 2007). There are three subtypes: PPAR-α, PPAR-δ and PPAR-γ (PPAR-γ1, 2 and 3), all of which are expressed in a tissue specific manner. Generally, PPARs heterodimerize with retinoid X receptors (RXRs) and, upon ligand binding and cofactor recruitment, increase the transcription rate of specific genes (Burstein 2005). PEA activates PPAR-α at low micromolar concentrations and thereby is involved in PPAR-α–mediated regulation of gene expression (LoVerme et al. 2005). Accordingly, genetic deletion of PPAR-α abolishes PEA's inflammatory effects in a CB_2-independent manner (LoVerme et al. 2005). When interpreting this particular study in light of others, one could conclude that there are both CB_2-sensitive and CB_2-insensitive components to PEA's anti-inflammatory and nociceptive actions (Calignano et al. 1998; LoVerme et al. 2005). However, one should also consider that the ligand-binding domains of PPARs are large and thus may exhibit promiscuous binding to an array of structurally related and unrelated chemicals (Kliewer et al. 1997). Accordingly, 2-AG, AEA, PEA and OEA (Lenman & Fowler 2007; Rockwell et al. 2006), and even some of their metabolites, exhibit comparable activities at PPARs (reviewed in O'Sullivan 2007). Thus, while it is clear that AEA and PEA can promote analgesia in a

synergistic manner, an important question remains: does PEA interact with a novel G-protein coupled CBR or a particular subtype of PPAR, or even both?

It is commonly accepted that a *bona fide* CBR should bind phytocannabinoids, and yet the prototypical phytocannabinoid, Δ^9-THC, activates PPAR-γ, another PPAR isoform, and increases PPAR-γ-regulated transcription, resulting in adipogenesis and vasorelaxation (O'Sullivan et al. 2006b; O'Sullivan et al. 2005). As a matter of fact, even CBD and the two synthetic cannabinoids WIN55,212-2 and CP55,940 also bind to PPAR-γ and increase transcriptional activity (O'Sullivan et al. 2006a). In regards to Δ^9-THC, while it has no significant activity at PPAR-α (Sun et al. 2007), it can induce vasorelaxation by producing NO and hydrogen peroxide, signaling molecules that require superoxide dismutase activation (O'Sullivan et al. 2005). Thus, while PEA might promote analgesia through PPAR-α, some phytocannabinoids might also mediate part of their analgesic response through PPAR-γ. Whether some of the PPAR subtypes should be included in the eCBSS and thus would represent novel players in the cannabinoid-mediated analgesia constitutes an intriguing possibility that warrants further investigation.

c.) Novel cannabinoid receptors regulating neurotransmission

The synapse is the fundamental unit of neural communication allowing for the transfer of chemical information from presynaptic terminals to their postsynaptic counterparts. The maintenance and tight regulation of this dynamic unit is crucial, since even small perturbations of this highly structured machinery may lead to dysfunctional neural communication often observed in neurological disease (reviewed in Beck & Yaari 2008). Most active synapses throughout the CNS contains functional elements of the eCBSS, and many laboratories have focused their attention on how the eCBSS regulates the efficacy of GABAergic and glutamatergic neurotransmission (Chevaleyre et al. 2006; Freund et al. 2003; Kreitzer & Regehr 2001; Lutz 2004; Maejima et al. 2001; Wilson et al. 2001). Because impaired eCBSS is implicated in several neurological diseases (Katona & Freund 2008; Kreitzer & Malenka 2007; Lastres-Becker et al. 2002a; Lastres-Becker et al. 2001; Lastres-Becker et al. 2002b; Lastres-Becker et al. 2002c; Pazos et al. 2008; Ramirez et al. 2005), the identification of novel receptors involved in the endocannabinoid (eCB)-mediated modulation of neurotransmission should allow for the development of better tools to understand the intricate role of the eCBSS in neurophysiology and treat neurological disease.

Novel CBR sites were first identified in brain homogenates. While AEA (EC_{50} = 3.6 μM) and WIN55,212-2 (EC_{50} = 1.8 μM) act this novel CBR by increasing [^{35}S]-GTPγS binding, this response does not involve CB_1 receptors since it is insensitive to SR141716A and reliably measured in $CB_1^{-/-}$ mice (Breivogel et al. 2001; Monory et al. 2002). Breivogel et al. (2001) reported that this novel CBR is expressed in brain stem, cortex, hippocampus, midbrain, and spinal cord, while being absent in the cerebellum and basal ganglia (Breivogel et al. 2001). Conversely, Monory et al. (2002) found this binding site in the cerebellum (Monory et al. 2002). However, it should be emphasized that these studies used two different $CB_1^{-/-}$ strains generated on distinct genetic backgrounds and that such differences could account for some of the discrepancies (Hoffman et al. 2005). While studies have reported CB_2 expression within specific brainstem neurons and in cerebellar granule neurons (Skaper et al. 1996; Van Sickle et al. 2005), this cannabinoid receptor subtype is unlikely to represent the aforementioned AEA- and WIN55,212-2-sensitive binding site since its activation is not blocked by SR144528 and unaffected by typical CB_2 receptor agonists, such as CP55,940, Δ^9-THC and HU-210 (Breivogel et al. 2001; Monory et al. 2002). In summary, these studies suggest the existence of a binding site sensitive to AEA and WIN55,212-2, resulting in G-protein activation, in a fashion that is clearly distinct from that of CB_1 and CB_2 receptors within the CNS.

Electrophysiological evidence suggests that this novel CBR regulates neurotransmission within the hippocampus, as first described by Hajos et al. by using $CB_1^{-/-}$ mice (Hajos et al. 2001). Specifically, WIN55,212-2 inhibited EPSCs but not IPSCs at the schaffer collateral synapse in the mouse hippocampal CA1 region (Hajos et al. 2001). This response was also present in rat hippocampal slices, where administration of the CB_1 antagonist AM251 abolishes the WIN55,212-2-mediated inhibition of IPSCs, but not EPSCs, indicating that EPSCs might be regulated by this novel CBR and not by CB_1 receptors (Hajos & Freund 2002). Remarkably, the vanilloid receptor antagonist capsezapine blocked the WIN55,212-2-mediated inhibition of EPSCs but not IPSCs (Hajos & Freund 2002). Considering the sensitivity of EPSCs to vanilloid receptor ligands, one could argue that this response is not mediated by a novel CBR but instead by a vanilloid receptor. While Hajos (2002) did argue against this possibility – citing a study that showed that WIN55,212-2 does not bind to TRP channels (Zygmunt et al. 1999) – more recent studies showed that a number of cannabinoids, including AEA, WIN55,212-2, and SR141716A, do actually bind to TRP channels and modulate TRP channel activity (De Petrocellis et al. 2008; Jeske et al. 2006; Patwardhan et al. 2006). Thus, the existence of a novel CBR (that is not the TRP channel) regulating neurotransmitter release in the hippocampus remains an open question. Note, however, that this situation is further complicated by a different result reported by Yoshida et al. who used juvenile $CB_1^{-/-}$ mice. In this study neither EPSCs nor IPSCs were inhibited by WIN55,212-2 or endogenously-released cannabinoids (Yoshida et al. 2002), suggesting that this novel CBR is absent in juvenile mice.

In summary, we still require better evidence for the existence of and better understanding for the role played by a novel CBR in regulating neurotransmitter release. This is particularly pertinent to the involvement of the eCBSS in critical neurological function, including learning and memory, and in neurological diseases such as Parkinson's and Huntington's disease. Thus, by determining the molecular identity and the fundamental role played by this novel CBR, this field of research will not only help solidify our understanding of how the eCBSS regulates neurotransmission, but also potentially unveil a valuable novel therapeutic target.

IV. GPR55 and S1P receptors: Novel Cannabinoid Receptors or independent lipid receptors?

a.) GPR55: a promising cannabinoid receptor candidate

The possibility that GPR55 might constitute the target responsible for some of the reported non-CB_1/CB_2 mediated effects has captured an increasing amount of attention. GPR55 was first identified in 1998 by performing homology searches of the amino acid sequences of known GPCRs using BLAST (basic alignment search tool) and publicly available databases (GenBank HighThroughput Genome and expressed sequence tag) (Sawzdargo et al. 1999). Its mRNA is expressed in caudate, putamen, hippocampus, thalamic nuclei, midbrain, spleen, intestine and fetal tissue as shown by Northern blot analysis and *in situ* hybridization (Sawzdargo et al. 1999). Two patents that followed these initial studies claimed that GPR55 represents a novel CBR, despite possessing only 13.5 % and 14.4% sequence homology to CB_1 and CB_2, respectively (reviewed by Baker et al. 2006). In more recent years, efforts from several independent laboratories aimed to verify if GPR55 indeed represents a novel CBR. These studies showed that cannabinoids, as well as LPI, activate this GPCR, with most studies agreeing that GPR55 activation causes $[Ca^{2+}]_i$ release by activation of IP_3 receptors (Henstridge et al. 2008; Lauckner et al. 2008; Oka et al. 2007; Waldeck-Weiermair et al. 2008). An interesting property of GPR55 activation is its potential to regulate neuronal excitability through $[Ca^{2+}]_i$ flux (Lauckner et al. 2008). Conversely, one study did not corroborate these results, for it showed that the cannabinoid-induced activation of GPR55

(using FLAG-tagged-GPR55 transiently transfected in HEK293 cells) does not lead to G_q-coupling to elicit the flux of $[Ca^{2+}]_I$; but rather leads to G_{13}-coupling, the latter resulting in RhoA, cdc42 and rac1 activation (Ryberg et al. 2007). One additional factor that could further complicate the comparison of these studies with the other ones is that Ryberg et al. utilized the FLIPR assay to measure $[Ca^{2+}]_i$ levels, which may not be as sensitive compared to ratiometric fluorescent dyes tested in single cells (Henstridge et al. 2008; Lauckner et al. 2008; Oka et al. 2007). Furthermore, it is well known that epitope tags can significantly influence the trafficking and downstream effector signaling of GPCRs, and thus native GPR55 receptors might exhibit differential coupling to signaling systems (Brothers et al. 2003).

Of note concerning all these studies, is the limited number of cannabinoids tested on GPR55, leaving its overall pharmacological profile relatively unexplored. More specifically, Oka et al (2007) states that GPR55 is an LPI receptor because low micromolar concentrations of cannabinoids do not bind (Oka et al. 2007), while other studies do show that cannabinoids, such as Δ^9-THC, do activate GPR55 albeit at higher concentrations (Lauckner et al. 2008). Although Henstridge et al. (2008) tested AEA and 2-AG (3–30 μM), as well as the synthetic cannabinoid CP55,940 (3 μM), and found no effect on $[Ca^{2+}]_i$ mobilization, this panel of cannabinoids remains limited in variety and dose. Moreover, since AEA and 2-AG are subject to hydrolysis at varying degrees depending on cell type and experimental conditions (Giuffrida et al. 2001), it might be important to include inhibitors of eCB hydrolysis so that the full efficacy of these labile lipids is preserved when testing their activity at GPR55.

Atypical cannabinoids, known to induce vasodilation, bind to GPR55 in low nanomolar concentrations (Ryberg et al. 2007), thus suggesting the possibility that GPR55 may constitute the long sought after endothelial receptor. Abn-CBD and O-1602, another atypical cannabinoid, both stimulate $[^{35}S]$-GTPγS binding in GPR55-expressing HEK293T cells. More importantly, however, the vasodilatory effect of abn-CBD is unchanged in mice lacking GPR55 expression when compared to wild-type control mice (Johns et al. 2007). Thus, it is likely that GPR55 is distinct from the endothelial receptor mediating the cannabinoid-induced vasodilatory effect.

Parallel studies investigated the intriguing notion that GPR55 may constitute the novel receptor responsible for some of the cannabinoid-mediated analgesic effects. As such, genetically modified mice lacking GPR55 were used to investigate the role of GPR55 in hyperalgesia associated with both neuropathic and inflammatory pain (Staton et al. 2007). Results indicate the GPR55 is involved in the regulation of various cytokine levels likely resulting in blunted inflammatory mechanical hyperalgesic responses in addition to the lack of mechanical hyperalgesic responses (Staton et al. 2007). This suggests that targeting GPR55 may be of therapeutic benefit in the regulation of pain, but it is likely that GPR55 will not be identified as the novel CBR mediating analgesia that we outlined above due to clear pharmacological discrepancies. Indeed, as reviewed in see section IIIb of this review, PEA is a unique compound that induces SR144528-sensitive analgesia, yet does not activate CB_2 receptors (LoVerme et al. 2005; Showalter et al. 1996). Interestingly, PEA is a high affinity ligand that activates $[^{35}S]$-GTPγS binding in GPR55-expressing HEK293 cells (Ryberg et al. 2007). However, SR144528 does not appear to influence GPR55 activation (Lauckner et al. 2008). Thus, GPR55 is a distinct receptor from the novel CBR mediating cannabinoin-induced analgesia.

The classification of GPR55 as either an LPI receptor (LPIR) or an additional CBR brings about three noteworthy speculations. First, it is possible that cannabinoids and lysophospholipids interact with the same GPCR, which would reiterate observations for

sphingosine-1-phosphate (S1P) at CB_1 (discussed in a following section of this review). Second, if one assumes that the deorphanization of a receptor depends on the identification of high affinity ligands, as many pharmaceutical companies do, then GPR55 is more likely to constitute a CBR since both patents and one peer-reviewed study reported that several cannabinoids activate GPR55-mediated GTPγS at low nanomolar concentrations (Ryberg et al. 2007). As mentioned previously, a novel CBR is generally defined as a receptor that binds phytocannabinoids; and GPR55 would fulfill this criterion since it is activated by Δ^9-THC, albeit at high concentrations (Lauckner et al. 2008). Third, it is interesting to consider the disparity between the pharmacological similarity that might exist between GPR55 and CBRs and the lack of amino acid sequence similarities, since GPR55 shares only 14% similarity with CB_1 and CB_2. This argument is further supported by results obtained when analyzing phylogenetic divergences of this family of GPCRs, a concept developed in a recent review by Brown (2007). Specifically, it is quite possible that although GPR55 lacks high sequence similarity to the CBRs, it may still express key amino acids that will allow its interaction with cannabinoid ligands. This logic was recently applied successfully with the past orphan receptors GPR23 and GPR92, now LPA_4 and LPA_5, in that although they are not closely phylogenetically related to the other LPARs, they still bind LPA with high affinity (Brown 2007). This suggests that although phylogenetic analysis might sometimes be a reliable tool to *identify* novel receptor subtypes, it is not a definitive method and pharmacology will often have the last word.

To conclude, while several studies have reported the pharmacology of some cannabinoids and lipids at GPR55, one of the most pressing and important question still remains open: does this receptor truly belong to the eCBSS? More specific compounds and reliable genetic tools will help the field answer this important question.

b.) S1P and its receptors: A clear link with the eCBSS

As stated above, identification of novel CBRs can be achieved either by using pharmacological tools or by comparing the amino acid sequences of CBRs to phylogenetically similar receptors, or sometimes by the combination of both approaches. While the beginning of this review focused on the pharmacological identification of novel CBRs, the following section discusses phylogenetic evidence supporting the existence of yet additional receptors that are engaged by cannabinoid compounds and thus could be considered novel CBRs. This evidence is based on sequence similarities between the CBRs and other lipid receptors, in particular the lysophospholipid receptors. The majority of our knowledge on the bioactivity of lysophospholipids is restricted to four main players: lysophosphatidic acid (LPA), lysophosphatidylcholine (LPC), sphingosylphosphorylcholine (SPC) and S1P. Here, we will focus on a possible link between CBRs and S1P receptors, the many subtypes of which are expressed throughout the body. A comparison of the literature available on the eCBSS and the S1P signaling systems indicates that these systems possess striking similarities among their respective receptors, ligands and transduction mechanisms, suggesting that they may converge or perhaps even superimpose.

Originally, sphingolipids were thought to only represent structural elements of cell membranes important for their stability and fluidity. However, several landmark studies had uncovered the signaling potential of S1P and this lipid is now recognized as a *bona fide* mediator of specific physiological functions, some of which have also been implicated in CBR signaling. Like other signaling lipids, including eCBs, the levels of S1P are tightly regulated by the balance between its synthesis and degradation. Sphingosine kinase (SPHK) is responsible for its *de novo* synthesis, while its degradation is controlled in a reversible manner by S1P-phosphatases (SPPs) and in an irreversible manner by S1P-lyase (Jo et al. 2008). There are two isoforms of SPHK, SPHK1 and SPHK 2, which are differentially expressed throughout the body and possess different levels of activity (Kohama et al. 1998;

Liu et al. 2000; Olivera et al. 1999). While low nanomolar concentrations of S1P are found in the intracellular space, much higher concentrations of S1P are found in the serum bound to albumin and other lipoproteins (Okajima 2002).

At the cell surface, S1P binds to and activates G-protein-coupled S1P receptors to elicit or regulate a wide range of biological functions, such as angiogenesis and immune functions (Jo et al. 2008; Lee et al. 1999b; Skoura et al. 2007), as well as cell proliferation and motility (Durand et al. 2006; LaMontagne et al. 2006; Lee et al. 1999a; Park et al. 2007). S1P receptors ($S1P_{1\text{-}5}$), formerly known as the endothelial differentiation gene (EDG) receptors ($EDG_{1,3,5,6,8}$), encompasses a class of GPCRs activated by the major sphingolipid metabolite S1P (Zondag et al. 1998). Interestingly, one report suggests that S1P might also constitute an endogenous ligand for GPR3, GPR6 and GPR12 (Uhlenbrock et al. 2002), but this report has not been followed up by other laboratories. All S1P receptors couple to $G_{i/o}$ and $G_{12/13}$, except $S1P_1$, which only couples to $G_{i/o}$. $S1P_2$ and $S1P_3$ receptors can also couple to G_q and G_s proteins while $S1P_4$ receptors can couple to G_s. Upon activation, all receptors activate MAPK, except $S1P_5$, which is associated with decreased MAPK phosphorylation (Ishii et al. 2004). Although older studies suggested that S1P might also be a second messenger that mobilizes calcium and regulates cell proliferation (Spiegel 1999; Zhang et al. 1991), its intracellular target remains to be identified and this concept remains to be confirmed.

An important study in the context of this review indicated that S1P analogs interact with CB_1 receptors (Paugh et al. 2006), which raises the exciting possibility that these two signaling systems might interact in ways that warrant further evaluation. Three lines of evidence favor this possibility. Phylogenetic analysis shows that S1P receptors share approximately 30% amino acid identity with CBRs (Figure 2). GPR3, GPR6, and GPR12 also exhibit high levels of homology with CBRs, averaging 28% amino acid identity (Table 2). Both S1PRs and CBRs are activated by endogenous lipid modulators that also share chemical and structural similarities (Figure 3). The last line of evidence – and in our opinion the most exciting – suggested a possible direct association and/or cross-talk between these signaling systems. Specifically, radioligand competition experiments that target CB_1 receptors stably expressed in CHO cells and HEK293 cells, as well as endogenously expressed CB_1 receptors expressed in mouse cerebellar homogenates, were performed. The results showed that low micromolar concentrations of the high affinity non-selective S1P receptor agonist, FTY720, and the endogenous lipid sphingosine, clearly competed for [^{3}H]-CP55,940 specific binding at CB_1 receptors (Paugh et al. 2006). Conversely, this effect was not observed when using CB_2 receptors, highlighting the specificity of these data. Two important findings result from this study. First, there is a clear pharmacological interaction between S1P receptor ligands and CB_1 receptors. Second, sphingosine is the first identified endogenous antagonist for CB_1 receptors as shown by performing GTPγS binding experiments (Paugh et al. 2006). In order to fully grasp the depth and relevance of these results, many basic questions still need to be answered, including whether the reverse scenario is true: do eCBs interact with S1P receptors? To our knowledge, an answer to this question has not been reported.

Additional studies focusing on either S1P or CBR signaling indicate further parallels between these systems. For example, several studies have shown therapeutic potential for cannabinoids to control neoangiogenesis and tumor vascularization (Blazquez et al. 2003; Casanova et al. 2003; Galve-Roperh et al. 2000; Sanchez et al. 2001) and similar therapeutic effects that has been shown for S1P (LaMontagne et al. 2006; Schmid et al. 2007). Another intriguing link was reported by Lee et al, (2000), since they showed that micromolar concentrations of S1P increases the cell motility of HUVEC in a PTX-sensitive manner (Lee et al. 2000). While this study directly concluded that this response was mediated by S1P

receptors, the authors did not consider the involvement of CB_1 receptors, even thought the latter are abundantly expressed by HUVEC (McCollum et al. 2007).

GPR3, GPR6 and GPR12, all three of which are engaged by S1P, share high amino acid similarity with CBRs (Table 2). These three GPCRs are thought to be constitutively active, stably increasing basal cAMP levels (Eggerickx et al. 1995; Kostenis 2004; Uhlenbrock et al. 2002). Note that GPR12 is grouped in this category of S1P lipid receptors despite its higher affinity toward another lysophospholipid, namely SPC (Uhlenbrock et al. 2002). GPR3, 6 and 12 are expressed at relatively high levels in the periphery and the CNS (Eidne et al. 1991; Heiber et al. 1995; Uhlenbrock et al. 2003), but subtle differences are found when considering their temporal and region specific expression patterns. For example, GPR3 is highly expressed in cerebral granule neurons to regulate neurite outgrowth (Tanaka et al. 2007), whereas GPR6 is expressed in striatopallidal medium spiny neurons where it is involved in instrumental learning (Lobo et al. 2007). With regards to GPR12, this receptor facilitates axonal regeneration by activating PKA and inhibiting Rho activation (Tanaka et al. 2007), a mechanism of likely importance for neural injury and development. Thus, here too, an intriguing link exists between S1P candidate receptors and CBRs.

In summary, evidence indicates that CBRs and S1P receptors share a high degree of sequence homology, are activated by chemically and structurally similar lipids and are implicated in the same biological processes, suggesting the possibility that these signaling systems may converge or even overlap at the receptor or signal transduction levels. This receptor duo is likely to dynamically and efficiently regulate key biological processes, and thus may represent a novel venue for therapeutic approaches.

V. Closing remarks

More than 30% of currently marketed drugs target G-protein coupled receptors (Wise et al. 2002), a statistic that emphasizes the importance of understanding GPCR physiology and molecular signaling. CB_1 and CB_2 receptors mediate many, but not all, of the biological effects produced by cannabinoid compounds; and the psychotropic effects associated with *C. sativa* and CB_1 receptor activation have diminished the enthusiasm for promoting the medicinal properties of this plant. Yet, the therapeutic benefit of this plant has helped define the significance of the eCBSS pathophysiological processes and has taught us much about how to target its components. The demonstration of the existence of novel CBRs has generated a new wave of interest in this field of research, one that could lead to pharmacological interventions that are devoid of the psychotropic and euphoric effects attributed to the activation of CB_1 receptors. Thus, the selective targeting of novel CBR could help regulate important pathophysiological processes linked to vasodilation and neurotransmission in both the periphery and the CNS.

However in order to unequivocally confirm that all of the aforementioned reviewed studies are indeed attributable to a novel CBR, their molecular entities must be identified and their pharmacology clearly defined. We believe that the pharmacological definition of the eCBSS might actually become more challenging since some of the criteria used to define a new CBR remain ambiguous: should a novel CBR share high amino acid sequence similarity to CB_1 and CB_2? Is it required that the novel CBR bind eCBs, phytocannabinoids or both? Must the receptor possess high affinity and efficacy for these compounds? How much of the selective targeting of these novel CBRs should induce specific physiological action within a therapeutic range and without side-effects? These are only some of the basic questions that must be addressed when identifying and defining a target as a novel CBR. Yet when thinking about how to answer these questions, we might have to revisit the definition of a CBR depending on the results that will be reported. Do we need a working definition that

will help us interpret the state of the current literature regarding receptor mediated non-CB_1/CB_2 observations, and if so, what should it be? Independent of these nomenclature considerations, exploitation of cannabinoid-based therapeutics will greatly benefit from the molecular identification and their precise pharmacological characterization of these novel CBRs. The burning question that we have is: which one of these receptors will be the first to unambiguously join the eCBSS?

Acknowledgments

This work was supported by our NIH grant DA021285. The authors wish to thank Eiron Cudaback, Eric Horne, William Marrs, Aaron Miller and Michelle Sexton, members of the Stella laboratory and Anatol Kreitzer (Gladstone Institute of Neurological Disease and Department of Physiology and Neurology University of California, San Francisco) for their critical reading and insights during the preparation of this manuscript.

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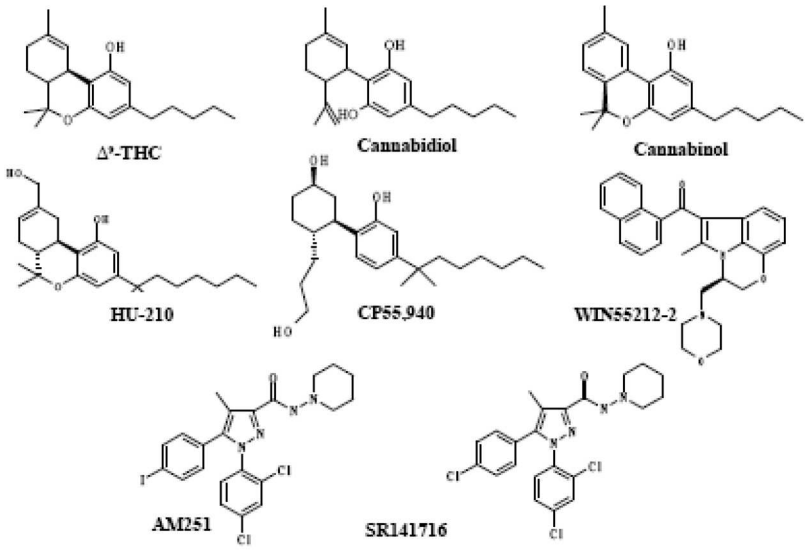

Figure 1. The structures of commonly used cannabinoids

The classical cannabinoids are Δ^9-THC, cannabidiol, cannabinol and the synthetic cannabinoid HU-210. CP55,940 is the prototypical non-classical cannabinoid and WIN55,212-2 is the prototypical aminoalkylindole. AM251 and SR141716 are both used as CB_1 antagonists.

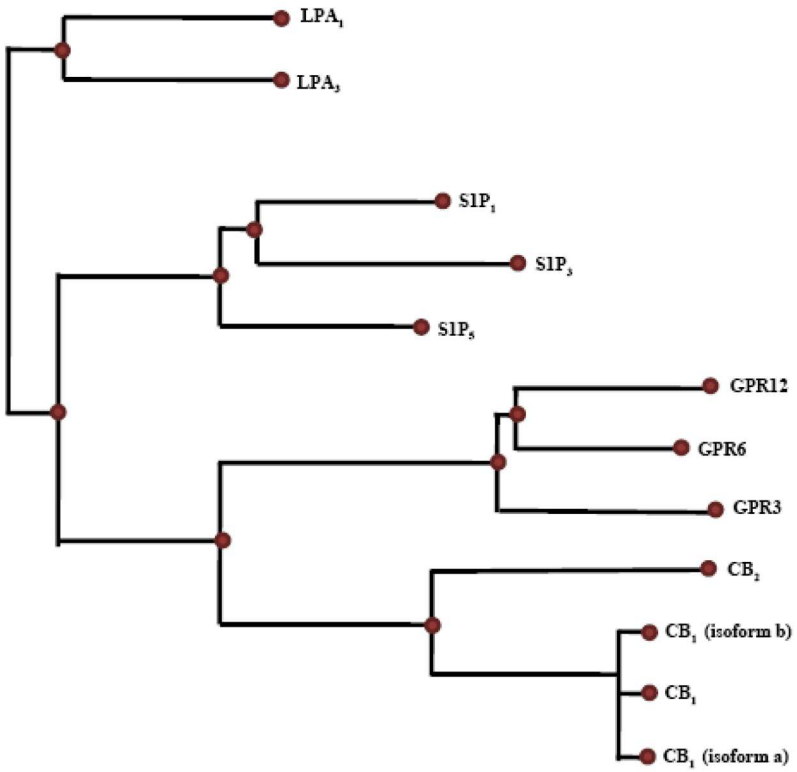

Figure 2. Phylogenetic tree analysis of GPCRs closely related to CB_1

Using the standard protein-protein BLAST (blastp) analysis, these related GPCRs share the highest amino acid sequence identities to CB_1.

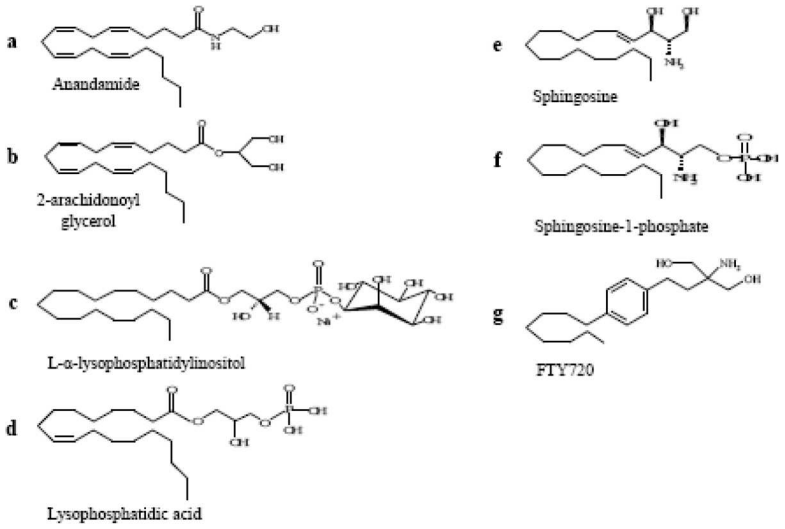

Figure 3. Structures of endogenous and synthetic lipids

The endogenous cannabinoids anandamide (a) and 2-arachidonoyl glycerol (b). The putative endogenous ligand for GPR55, lysophosphatidylinositol (c). Lysophosphatidic acid (d), the endogenous lipid for LPA receptors. The endogenous lipids for S1P receptors sphingosine (e) and sphingosine-1-phosphate (f). The synthetic S1P receptor ligand FTY720 (g).

Table 1

Reported pharmacology of cannabinoid compounds referenced in this review.

Compound	CB_1	K_i (nM)	Reference	EC_{50} (nM)	Reference	CB_2	K_i (nM)	Reference	EC_{50} (nM)	Reference
Phytocannabinoids										
Δ^9-tetrahydrocannabinol (Δ^9-THC)	partial agonist	3.9	Rinaldi-Carmona *et al.*, 1994[b,2]	70.9	Burkey *et al.*, 1997[c,2]	partial agonist	28.3	Griffin *et al.*, 2000[b,3]	22,000*	Schatz *et al.*, 1996[c,4]
		53.3	Felder *et al.*, 1995[a,3]	87	Breivogel *et al.*, 1998[b,2]		27.3	Griffin *et al.*, 2000[c,3]	>1,000	Bayewitch *et al.*, 1996[d,3]
		40.7	Showalter *et al.*, 1996[a,3]	13	Bayewitch *et al.*, 1996[d,3]		44.9	Griffin *et al.*, 2000[a,3]	1,000*	Rhee *et al.*, 1997[b,3]
		80.3	Rhee *et al.*, 1997[b,3]	35	Bayewitch *et al.*, 1996[b/c, 3]		32.2	Rhee *et al.*, 1997[b,3]	>1,000*	Iwamura *et al.*, 2001[a,3]
		66.5	Rhee *et al.*, 1997[b,2]	15, 10	Bouaboula *et al.*, 1995[a,3]		40	Munro *et al.*, 1993[a,3]	~100	Iwamura *et al.*, 2001[c,3]
		13.5	Iwamura *et al.*, 2001[b,2]	15	Gomez del Pulgar *et al.*, 2000[d,3]		36	Showalter *et al.*, 1996[a,3]		
		8.3	Iwamura *et al.*, 2001[c,2]	13.5	Matsuda *et al.*, 1990[b,3]		11.8	Schatz *et al.*, 1996[c,4]		
		5.1	Iwamura *et al.*, 2001[a,3]	11	Rhee *et al.*, 1997[b,3]		40	Bayewitch *et al.*, 1996[d,3]		
		3.9	Bouaboula *et al.*, 1995[a,3]	16.5	Felder *et al.*, 1995[a,3]		75.3	Felder *et al.*, 1995[a,3]		
		1.6	Devane *et al.*, 1988[b,2]				15.8	Shire *et al.*, 1996[c,3]		
							21.4	Shire *et al.*, 1996[a,3]		
Cannabidiol (CBD)	inverse agonist	4,350	Showalter *et al.*, 1996[a,3]			inverse agonist	2860	Showalter *et al.*, 1996[a,3]		
		> 500	Devane *et al.*, 1988[b,2]				> 1,000	Facci *et al.*, 1995[e,3]		
		4,900	Thomas *et al.*, 2004[c,2]				38,000	Munro *et al.*, 1993[a,3]		
							4,200	Thomas *et al.*, 2004[e,3]		

Compound	CB_1	K_i (nM)	Reference	EC_{50} (nM)	Reference	CB_2	K_i (nM)	Reference	EC_{50} (nM)	Reference
Endocannabinoids										
Anandamide (AEA)	partial agonist	252	Mechoulam *et al.*, 1995[d,3]	846	Burkey *et al.*, 1997[c,2]	partial agonist	581	Mechoulam *et al.*, 1995[d,3]	260	Gonsiorek *et al.*, 2000[a,3]
		543	Felder *et al.*, 1995[a,3]	100	Berglund *et al.*, 1998[b,2]		1940	Felder *et al.*, 1995[a,3]	> 10,000	Hillard *et al.*, 1999[a,3]
		78.2	Khanolkar *et al.*, 1996[b,2]	390	Breivogel *et al.*, 1998[b,2]		1926	Khanolkar *et al.*, 1996[b,2]	10,430	Leggett *et al.*, 2004[b,2]
		89	Showalter *et al.*, 1996[a,3]	3,000	Pinto *et al.*, 1994[b,3]		371	Showalter *et al.*, 1996[a,3]	957	Felder *et al.*, 1995[a,3]
		61	Lin *et al.*, 1998[b,2]	540	Vogel *et al.*, 1993[b,3]		1930	Lin *et al.*, 1998[b,4]		
		71.9	Hillard *et al.*, 1999[b,2]	6,000	Savinainen *et al.*, 2003[b,2]		279	Hillard *et al.*, 1999[b,4]		
		428	Leggett *et al.*, 2004[b,2]	> 1,000	Sugiura *et al.*, 1996[c/d, 3]		>10,000	Griffin *et al.*, 2000[b,3]		
		78	Abadji *et al.*, 1994[b,2]	10,400	Legget *et al.*, 2004[b,2]		1480	Griffin *et al.*, 2000[c,3]		
		89	Adams *et al.*, 1995[b,2]	69	Steffens *et al.*, 2005[b,2]		306	Griffin *et al.*, 2000[a,3]		
		2320	Adams *et al.*, 1998[b,2]	322	Felder *et al.*, 1995[a,3]		33	Facci *et al.*, 1995[e,3]		
		200	Steffens *et al.*, 2005[a,2]	444	Hillard *et al.*, 1999[a,3]		~3	Munro *et al.*, 1993[a,3]		
							326	Shire *et al.*, 1996[c,3]		
							320	Shire *et al.*, 1996[a,3]		
2-arachidonoylglycerol (2-AG)	agonist	472	Mechoulam *et al.*, 1995[d,3]	85	Pinto *et al.*, 1994[b,3]	agonist	1400	Mechoulam *et al.*, 1995[d,3]	122	Gonsiorek *et al.*, 2000[a,3]
		58.3	Ben-Shabat *et al.*, 1998[d,3]	1,300	Gonsiorek *et al.*, 2000[a,3]		145	Ben-Shabat *et al.*, 1998[d,3]	238	Shoemaker et al., 2005[a, 3]
		> 10,000	Steffens *et al.*, 2005[a,2]	1,000	Savinainen *et al.*, 2003[b,2]				12.4, 238	Shoemaker et al., 2005[a, 3]

Compound	CB_1	K_i (nM)	Reference	EC_{50} (nM)	Reference	CB_2	K_i (nM)	Reference	EC_{50} (nM)	Reference
		10,720	Steffens *et al.*, 2005[b,2]	150	Sugiura *et al.*, 1996[c/d, 3]					
				12.6	Steffens *et al.*, 2005[a,2]					
Putative Endocannabinoids										
Palmitoylethanolamide (PEA)	no activity	–	Skaper *et al.*, 1996[c,2]			no activity	> 10,000	Showalter *et al.*, 1996[a,3]		
		–	Felder *et al.*, 1993[a,3]				1	Facci *et al.*, 1995[e,3]		
oleamide	limited activity	1,140	Leggett *et al.*, 2004[b,2]	1,640	Leggett *et al.*, 2004[b,2]	no activity	> 100,000	Leggett *et al.*, 2004[a,3]		
		2,630	Leggett *et al.*, 2004[b,2]							
		8,130	Leggett *et al.*, 2004[a,3]							
N-arachidonoyl-L-serine (ARA-S)	no activity	> 10,000	Milman *et al.*, 2006[c,2]			no activity	> 30,000	Milman *et al.*, 2006[b,3]		
Antagonists										
SR141716A (SR1)	antagonist	5.6	Rinaldi-Carmona *et al.*, 1994[a,3]	0.8	Landsman *et al.*, 1997[a,3]	limited activity	>1,000	Rinaldi-Carmona *et al.*, 1994[b,2]	1,000	MacLennan *et al.*, 1998[a, 3]
		1.98	Rinaldi-Carmona *et al.*, 1994[b,2]	~ 3	Bouaboula *et al.*, 1997[a,3]		973	Felder *et al.*, 1995[a,3]	>1,000	Felder *et al.*, 1995[a,3]
		11.8	Felder *et al.*, 1995[a,3]	10	MacLennan *et al.*, 1998[a,3]		13,200	Felder *et al.*, 1998[d,3]		
		11.8	Felder *et al.*, 1998[d,3]	2.1	Legget *et al.*, 2004[b,2]		702	Showalter *et al.*, 1996[a,3]		
		12.3	Showalter *et al.*, 1996[a,3]	143	Felder *et al.*, 1995[a,3]		514	Ruiu *et al.*, 2003[c,4]		
		1.8	Ruiu *et al.*, 2003[c,2]				47	Shire *et al.*, 1996[c,3]		
		47	Adams *et al.*, 1998[b,2]				38	Shire *et al.*, 1996[a,3]		
		9	Hoi *et al.*, 2007[b,2]				2,496	Lunn *et al.*, 2006[a,3]		

Compound	CB_1	K_i (nM)	Reference	EC_{50} (nM)	Reference	CB_2	K_i (nM)	Reference	EC_{50} (nM)	Reference
AM251	antagonist	700	Gatley *et al.*, 1996[c,2]	56	Chambers *et al.*, 2007[a,3]	limited activity	124	Chambers *et al.*, 2007[a,3]		
		7.5	Lan *et al.*, 1999[b,2]							
		3.4	Chambers *et al.*, 2007[a,3]							
SR144528 (SR2)	limited activity	437	Rinaldi-Carmona *et al.*, 1998[a,3]	> 10,000	Ross *et al.*, 1999[a,3]	antagonist	0.6	Rinaldi-Carmona *et al.*, 1998[a,3]	3, 18	Bouaboula *et al.*, 1998[a,3]
		305	Rinaldi-Carmona *et al.*, 1998[b,4]				0.3	Rinaldi-Carmona *et al.*, 1998[b,4]	~3	Gansiorek *et al.*, 2006[a,3]
		> 10,000	Ross *et al.*, 1999[a,3]				0.3	Griffin *et al.*, 2000[b,3]	~30	Iwamura *et al.*, 2001[a,3]
		27.6	Iwamura *et al.*, 2001[b,2]				0.1	Griffin *et al.*, 2000[c,3]	~10	Iwamura *et al.*, 2001[c,3]
		20.1	Iwamura *et al.*, 2001[c,2]				0.3	Griffin *et al.*, 2000[a,3]	23	Lunn *et al.*, 2006[a,3]
		50.3	Iwamura *et al.*, 2001[a,3]				0.2	Iwamura *et al.*, 2001[b,2]		
		70	Ruiu *et al.*, 2003[c,2]				0.04	Iwamura *et al.*, 2001[c,2]		
							1.99	Iwamura *et al.*, 2001[a,3]		
							0.28	Ruiu *et al.*, 2003[c,4]		
							14.9	Lunn *et al.*, 2006[a,3]		
							4.2	Hillard *et al.*, 1999[b,4]		
Synthetic Cannabinoids										
HU-210	agonist	0.06	Felder *et al.*, 1995[a,3]	2.26	Burkey *et al.*, 1997[c,2]	agonist	0.5	Felder *et al.*, 1995[a,3]	1.96	Gonsiorek *et al.*, 2000[a,3]
		0.7	Showalter *et al.*, 1996[a,3]	0.55	Griffin *et al.*, 1998[b,2]		0.2	Showalter *et al.*, 1996[a,3]	1	Bayewitch *et al.*, 1995[a,3]

Compound	CB_1	K_i (nM)	Reference	EC_{50} (nM)	Reference	CB_2	K_i (nM)	Reference	EC_{50} (nM)	Reference
				0.2	Felder *et al.*, 1995[a,3]		3.2	Lunn *et al.*, 2006[a,3]	0.58	Felder *et al.*, 1995[a,3]
									2.9	Lunn *et al.*, 2006[a,3]
CP55,940 (CP)	agonist	1.4	Rinaldi-Carmona *et al.*, 1994[b]	61.7	Burkey *et al.*, 1997[c,2]	agonist	1.4	Rinaldi-Carmona *et al.*, 1994[b]	3	MacLennan *et al.*, 1998[a,3]
		3.7	Felder *et al.*, 1995[a,3]	100	Selley *et al.*, 1996[b,2]		2.6	Felder *et al.*, 1995[a,3]	2.6, 5.3	Shoemaker et al., 2005[a,3]
		0.6	Showalter *et al.*, 1996[a,3]	17.6	Griffin *et al.*, 1998[b,2]		0.7	Showalter *et al.*, 1996[a,3]	5, 8	Bouaboula *et al.*, 1998[a,3]
		0.5	Hillard *et al.*, 1999[b,2]	6.6	Breivogel *et al.*, 1998[b,2]		2.8	Hillard *et al.*, 1999[b,4]	~100	Gansiorek *et al.*, 2006[a,3]
		5	Ross *et al.*, 1999[a,3]	19	MacLennan *et al.*, 1998[a,3]		1.8	Ross *et al.*, 1999[a,3]	1.6	Munro *et al.*, 1993[a,3]
		9	Adams *et al.*, 1998[b,2]	75	Savinainen *et al.*, 2003[b,2]		0.6	Griffin *et al.*, 2000[b,3]	2.89	Felder *et al.*, 1995[a,3]
		0.7	Bouaboula *et al.*, 1995[a,3]	3.4, 1.2	Bouaboula *et al.*, 1995[a,3]		0.7	Griffin *et al.*, 2000[c,3]	2.89	Hillard *et al.*, 1999[a,3]
		0.8	Compton *et al.*, 1993[b,2]	0.87	Matsuda *et al.*, 1990[b,3]		0.9	Griffin *et al.*, 2000[a,3]	2.9	Ross *et al.*, 1999[a,3]
		0.4	Hoi *et al.*, 2007[b,2]	1.83	Felder *et al.*, 1995[a,3]		1.9	Schatz *et al.*, 1996[c,4]		
				2.6	Hillard *et al.*, 1999[a,3]		5.6	Shire *et al.*, 1996[c,3]		
				2.6	Ross *et al.*, 1999[a,3]		3.2	Shire *et al.*, 1996[a,3]		
WIN55,212-2 (WIN-2)	agonist	35	Rinaldi-Carmona *et al.*, 1994[b,2]	180	Selley *et al.*, 1996[b,2]	agonist	16.2	Rinaldi-Carmona *et al.*, 1994[b]	2	MacLennan *et al.*, 1998[a,3]
		62.3	Felder *et al.*, 1995[a,3]	151.1	Griffin *et al.*, 1998[b,2]		3.3	Felder *et al.*, 1995[a,3]	3.7	Munro *et al.*, 1993[a,3]
		1.9	Showalter *et al.*, 1996[a,3]	160	Breivogel *et al.*, 1998[b,2]		4.9	Shire *et al.*, 1996[c,3]	0.41	Felder *et al.*, 1995[a,3]
		4.4	Hillard *et al.*, 1999[b,2]	617	MacLennan *et al.*, 1998[a,3]		0.3	Showalter *et al.*, 1996[a,3]	~1	Iwamura *et al.*, 2001[a,3]

Compound	CB_1	K_i (nM)	Reference	EC_{50} (nM)	Reference	CB_2	K_i (nM)	Reference	EC_{50} (nM)	Reference
		0.14	Iwamura *et al.*, 2001[b,2]	210	Sugiura *et al.*, 1996[c/d, 3]		1.2	Hillard *et al.*, 1999[b,4]	~1	Iwamura *et al.*, 2001[c,3]
		0.4	Iwamura *et al.*, 2001[c,2]	441	Houston *et al.*, 1998[b,2]		10.4	Griffin *et al.*, 2000[b,3]	4.6	Lunn *et al.*, 2006[a,3]
		9.9	Iwamura *et al.*, 2001[a,3]	40, 14	Bouaboula *et al.*, 1995[a,3]		9.5	Griffin *et al.*, 2000[c,3]		
		485	Bouaboula *et al.*, 1995[a,3]	40	Rinaldi-Carmona *et al.*, 1994[b,2]		1.2	Griffin *et al.*, 2000[a,3]		
		1.6	Skaper *et al.*, 1996[c,2]	24	Felder *et al.*, 1995[a,3]		1.3	Iwamura *et al.*, 2001[b,2]		
		38	Houston *et al.*, 1998[b,2]	~ 100	Iwamura *et al.*, 2001[a,3]		0.6	Iwamura *et al.*, 2001[c,2]		
							0.3	Iwamura *et al.*, 2001[a,3]		
							6.8	Schatz *et al.*, 1996[c,4]		
							4.9	Shire *et al.*, 1996[a,3]		
Abnormal cannabidiol (abn-CBD)	no activity	> 10,000	Showalter *et al.*, 1996[a,3]			no activity	> 10,000	Showalter *et al.*, 1996[a,3]		
		> 30,000	Offertaler *et al.*, 2003[c,2]				> 30,000	Offertaler *et al.*, 2003[c,3]		
O-1918	no activity	> 30,000	Offertaler *et al.*, 2003[c,2]			no activity	> 30,000	Offertaler *et al.*, 2003[c,3]		
Methanandamide (mAEA)	agonist	20	Abadji *et al.*, 1994[b,2]	> 5,000	Savinainen *et al.*, 2003[b,2]	partial agonist	868	Goutopoulos *et al.*, 2001[c,4]		
		28	Goutopoulos *et al.*, 2001[b,2]	1,400	Berglund *et al.*, 1998[b,2]		868	Lin *et al.*, 1998[b,4]		
		20	Khanolkar *et al.*, 1996[b,2]	~ 160	Breivogel *et al.*, 1998[b,2]					
		17.9	Lin *et al.*, 1998[b,2]	165	Pinto *et al.*, 1994[b,3]					
other compounds										
VSN16	no activity	> 300,000	Hoi *et al.*, 2007[b,2]							

A variety of species were utilized across studies thus species are distinguished by the following annotations:

[a] human,

[b] rat,

[c] mouse,

[d] not specified,

[e] other

A variety of preparations were utilized across studies thus are annoted by the following:

[1] brain primary cultured neurons,

[2] brain homogenate,

[3] cell line,

[4] spleen

EC_{50} values were determined by using various biochemical techniques to measure: cAMP, GTPgS activation, MAPK activation, Akt activation and calcium

[*] not EC_{50}

Table 2

Compared sequence identities of lysophospholipid receptors and cannabinoid receptors.

	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$	LPA_1	LPA_2	LPA_3	LPA_4	LPA_5	GPR3	GPR6	GPR12
CB_1	30%	28%	29%	32%	25%	27%	25%	26%	28%	NS	28%	32%	31%
CB_2	29%	26%	26%	24%	NS	27%	26%	26%	22%	NS	26%	27%	26%

Analysis was performed using Blast 2 sequences software on the amino acid sequences of the human isoforms of the listed receptors (Tatusova & Madden 1999).

Pharmacol Ther. Author manuscript; available in PMC 2013 June 18.

Table 3

Pharmacological identification of putative cannabinoid receptors.

Compound	Tissue of observed response	Pharmacology	Concentration tested	Reference
Phytocannabinoids				
Δ^9-tetrahydrocannabinol	mesenteric artery (G3)	↑ vasodilation	EC_{50}= 6.6 μM	O'Sullivan *et al.*, 2005[b]
	cerebral cortex	↑ radioligand binding	1 μM	Qureshi *et al.*, 1998[b, 2]
Cannabidiol	mesenteric artery	↓ vasodilation	10 μM	Jarai *et al.*, 1999[c]
Endocannabinoids				
anandamide	aortic rings	↑ vasodilation	k_i= 10,000 nM	Herradon *et al.*, 2007[b]
	mesenteric artery	↑ vasodilation	EC_{50}= 5.4 μM	O'Sullivan *et al.*, 2005[b]
	mesenteric artery	↑ vasodilation	ED_{50}= 79 nM	Wagner *et al.*, 1999[b]
	periphery (*in vivo*)	↑analgesia	10–30 mg/kg	Jaggar *et al.*, 1998[b]
	whole brain	↑GTPγS binding	EC_{50}= 3.6 μM	Breivogel *et al.*, 2001[c, 2]
	whole brain	↑GTPγS binding	EC_{50}= 10 μM	Di Marzo *et al.*, 2000[c, 2]
	whole animal (*in vivo*)	↑catalepsy	3, 10 mg/kg (IV)	Di Marzo *et al.*, 2000[c]
	cerebellum	↑GTPγS binding	EC_{50}= 4.9 μM	Monory *et al.*, 2002[c, 2]
	cerebral cortex	↑ radioligand binding	1 μM	Qureshi *et al.*, 1998[b, 2]
Putative Endocannabinoids				
palmitoylethanolamide	periphery (*in vivo*)	↑analgesia	30 μg	Calignano *et al.*, 1998[c]
	periphery (*in vivo*)	↑analgesia	10–30 mg/kg	Jaggar *et al.*, 1998[b]
N-arachidonoyl-L-serine	mesenteric artery	↑ vasodilation	EC_{50}=550 nM	Milman *et al.*, 2006[b]
	abdominal aorta	↑ vasodilation	EC_{50}=1,200 nM	Milman *et al.*, 2006[b]
oleamide	small mesenteric artery	↑ vasodilation	k_i= 1,200 nM	Hoi *et al.*, 2007[b]
Antagonists				
SR141716A	mesenteric artery	↓ vasodilation	1μM	Jarai *et al.*, 1999[c]
	hippocampus	blocks WIN55212-2-mediated inhibition of EPSCs	1 μM	Hajos *et al.*, 2001[c]
Synthetic Cannabinoids				
HU-210	mesenteric artery	↑ vasodilation	> 2,600 nM	Wagner *et al.*, 1999[b]

Compound	Tissue of observed response	Pharmacology	Concentration tested	Reference
CP55,940	cerebral cortex	↑ radioligand binding	k_d = 0.97 nM (cytosolic)	Qureshi *et al.*, 1998[b, 2]
			k_d = 3.3 nM (cytosolic)	
			k_d = 1.5 nM (membrane)	
WIN55,212-2	whole brain	↑GTPγS binding	EC_{50}= 1.8 μM	Breivogel *et al.*, 2001[c, 2]
	hippocampus	↓ EPSCs	1 μM	Hajos *et al.*, 2001[c]
	cerebellum	↑GTPγS binding	EC_{50}= 1.78 μM	Monory *et al.*, 2002[c, 2]
abnormal cannabidiol	small mesenteric artery	↑ vasodilation	k_i= 1,000 nM	Ho *et al.*, 2003[b]
	HUVEC	↑ migration	30 μM	Mo *et al.*, 2004[a]
	mesenteric artery	↑ vasodilation	EC_{50}= 5.6 μM	Offertaler *et al., 2003*[b]
O-1918	aortic endothelial	↓ vasodilation	not specified	McCollum *et al.*, 2007[e]
	HUVEC	↓ migration	30 μM	Mo *et al.*, 2004[a]
	mesenteric artery	↓ vasodilation	10 μM	Offertaler *et al., 2003*[b]
methanandamide	mesenteric artery	↑ vasodilation	10μg/kg	Jarai *et al.*, 1999[c]
	aortic endothelial	↑ vasodilation	EC_{50}= 9.4 nM	McCollum *et al.*, 2007[e]
	mesenteric artery	↑ vasodilation	ED_{50}= 286 nM	Wagner *et al.*, 1999[b]
other compounds				
VSN16	mesenteric artery	↑ vasodilation	k_i= 88 nM	Hoi *et al.*, 2007[b]
CPZ	hippocampus	blocks WIN55212-2-mediated inhibition of EPSCs	10 μM	Hajos *et al.*, 2002[b]

A variety of species were utilized across studies thus species are distinguished by the following annotations:

[a] human,

[b] rat,

[c] mouse,

[d] not specified,

[e] other

A variety of preparations were utilized across studies thus are annoted by the following:

[1] brain primary cultured neurons,

[2] brain homogenate,

[3] cell line,

[4] spleen

Abbreviations: HUVEC (human umbilical vein endothelial cells); EPSCs (excitatory post synaptic currents)