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Cannabinoid Receptors and Endocannabinoids: Evidence for New Players

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ABSTRACT

It is now well established that the psychoactive effects of *Cannabis sativa* are primarily mediated through neuronal CB1 receptors, while its therapeutic immune properties are primarily mediated through CB2 receptors. Two endocannabinoids, arachidonoylethanolamide and 2-arachidonoylglycerol, have been identified, their action on CB1 and CB2 thoroughly characterized, and their production and inactivation elucidated. However, many significant exceptions to these rules exist. Here we review the evidence suggesting that cannabinoids can modulate synaptic transmission, the cardiovascular system, and the immune system through receptors distinct from CB1 and CB2, and that an additional "independent" endocannabinoid signaling system that involves palmitoylethanolamide may exist.

KEYWORDS: drug of abuse, cannabinoids, marijuana, receptor

INTRODUCTION

Cannabis sativa, also known as marijuana or hashish depending on the particular preparation, is both a widespread illegal drug of abuse and a well-recognized medicinal plant.[1,2] A current challenge in pharmacology is to increase our understanding of the molecular mechanisms underlying the abuse property of *C sativa* in order to develop means to prevent it. Another challenge is to isolate the bioactive components that impart the medicinal properties ascribed to this plant with the goal of developing novel, cannabinoid-based therapeutics devoid of adverse effects. After approximately 40 years of research, we have a much clearer understanding of the pharmacology and molecular mechanisms mediating the bioactivity of plant-derived cannabinoid compounds, the phytocannabinoids, and are getting much closer to overcoming these 2 challenges.

C sativa contains ~60 phytocannabinoids, a handful of which are bioactive as defined by their ability to specifically interact with membrane-associated receptors, the cannabinoid receptors. The best-known phytocannabinoid is Δ^9-tetrahydrocannabinol (THC),[3] which is thought to mediate most—if not all—of the psychotropic and addictive properties of *C sativa*.[4] Recent evidence suggest that some of the antiinflammatory properties of *C sativa* may be accounted for by cannabinol (CBN) and cannabidiol (CBD), 2 nonpsychotropic phytocannabinoids that constitute promising lead compounds to develop cannabinoid-based antiinflammatory medicines.[5,6] Thus, while some might focus on THC to study the psychotropic and addictive properties of *C sativa*, others might focus on CBN and CBD as a means to develop cannabinoid-based antiinflammatory therapeutics devoid of adverse effects. Here we will review our current understanding of the cannabinoid receptors that mediate the effects of cannabinoids and provide examples of the biological functions regulated by them, with a special emphasis on those receptors that have been pharmacologically characterized and yet still remain to be cloned. We will also review our current understanding of the endogenous cannabinoids, the endocannabinoids, with a special emphasis on palmitoylethanolamide (PEA), whose production and inactivation can occur independently of other endocannabinoids, and whose biological effects are mediated by interactions with one of the novel cannabinoid receptors.

Cannabinoids Activate at Least 5 Distinct Cannabinoid Receptors

Two cannabinoid receptors, CB1 and CB2, have been identified by molecular cloning and are unambiguously established as mediators of the biological effects induced by cannabinoids, either plant derived, synthetic, or endogenously produced. CB1 and CB2 are 7 transmembrane Gi/o-coupled receptors that share 44% protein identity and display different pharmacological profiles and patterns of expression,[7,8] a dichotomy that provides a unique opportunity to develop pharmaceutical approaches. Studies performed with $CB1^{-/-}$ and/or $CB2^{-/-}$ mice identified additional cannabinoid receptors, 3 of which will be discussed below.

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CB1 Receptors

The vast majority of CB1 receptors are expressed in the central nervous system (CNS), and their distribution in this tissue has been documented in detail using highly specific antibodies and $CB1^{-/-}$ tissue controls. The current picture depicts abundant presynaptic expression in the adult mammalian brain.[9] In some cases, these receptors are also present on the dendrites and soma of neurons,[10] although at lower levels and frequency than their presynaptic counterparts. CB1 receptors are also expressed at low levels by various astrocytes, oligodendrocytes, and neural stem cells.[11-13] To the best of our knowledge, expression of CB1 receptors in situ by ependymal cells and/or microglia has not been reported.

CB1 receptors couple to Gi/o proteins and, under specific conditions, also to Gs proteins (only when other Gi/o protein-coupled receptors are concomitantly activated).[14,15] By coupling to Gi/o proteins, CB1 receptors regulate the activity of many plasma membrane proteins and signal transduction pathways, including ion channels, enzymes producing cyclic nucleotide second messengers, and various kinases. Thus, depending on the coupling and cell type expressing CB1 receptors, cannabinoids may regulate distinct cell functions. For example, activation of presynaptic CB1 receptors inhibits N-type calcium channels, thus reducing synaptic transmission.[9] It is likely that THC induces most—if not all—of its acute cognitive and intoxicating effects through this molecular mechanism.[16] Whether THC produces its effect by partially activating CB1 receptors or antagonizing the action of endocannabinoids on this receptor remains an open question.[17,18] Activation of CB1 receptors expressed on the somata of neurons increases Erk activity and induces brain-derived neurotrophic factor (BDNF) expression.[10] It is likely that the neuroprotective properties of cannabinoids are in part mediated through this mechanism. Recent evidence shows that CB1 receptor may also control the fate of neural stem cells, the outgrowth of neurites and the formation of functional synapses, emphasizing the importance of this receptor in the remodeling of neuronal networks.[13,19-21] Note that blood-derived leukocytes also express CB1 receptors,[22] suggesting that under neuropathological conditions, whereby the blood brain barrier is disrupted, cannabinoids accumulating in the CNS likely activate CB1 receptors expressed by invading leukocytes and thereby modulate the development of neuroinflammation.[23-25]

CB2 Receptors

Under nonpathological conditions, CB2 receptors are primarily expressed by leukocytes (with a rank order of B cells > natural killer [NK] cells >> monocytes/macrophages > neutrophils > $CD8^+$ T cells > $CD4^+$ T cells). Furthermore, experiments performed with $CB2^{-/-}$ mice show that cannabinoids engaging these receptors lead to changes in immune responses, particularly at the level of macrophage-induced helper T-cell activation.[8,22,26] CBN is more efficacious at CB2 receptors than THC,[27] making this phytocannabinoid more likely to account for the antiinflammatory properties of *C sativa*. For example, activation of CB2 receptors inhibits the ability of macrophages to process antigens and prime helper T cells[26,28-31] and at high concentrations may induce immune cell apoptosis.[32]

While many laboratories have reported the absence of CB2 receptor expression in healthy brain,[8,22,33-36] a new study has found that neurons located in specific brain structures, such as the brain stem, express detectable levels of CB2 mRNA and functional receptors, the activation of which is thought to regulate emesis.[37] Furthermore, while resting microglia present in healthy CNS tissue express few—if any—CB2 receptors, activated microglial cells, such as those found in mouse models of multiple sclerosis and Alzheimer's disease, express significant levels of CB2 receptors.[38,39] This result is not surprising since pathological conditions are known to induce CB2 receptor expression in leukocytes.[36] Microglial cells in culture, which by default are considered chronically activated, also express CB2 receptors, particularly at the leading edges of lamellipodia, protrusions involved in cell migration.[40] Accordingly, application of CB2 agonists induces cell migration, a response that can be extended to other CB2-expressing cells.[41-43] Furthermore, several laboratories have shown that CB2 agonists reduce the release of cytotoxins by immune cells and increase their proliferation rate.[44-47] Taken together, these studies suggest that the ability of CB2-selective compounds to reduce inflammation might be caused by an increase in proliferation and recruitment of immune cells, in particular a population of immune cells that do not release detrimental mediators and thus could be involved in the immune-mediated repair of damaged tissue. Again, blood-derived leukocytes expressing CB2 receptors and invading the CNS via a disrupted blood brain barrier are likely to be activated by cannabinoids accumulating in the CNS and to modulate the development of neuroinflammation. Whether astrocytes and endothelial cells express CB2 receptors remains controversial as control experiments using $CB2^{-/-}$ tissue have yet to be performed.

Similar to CB1 receptors, CB2 receptors also couple to Gi/o proteins and regulate the activity of signal transduction pathways, including enzymes that produce cyclic nucleotide second messengers and various kinases. Accordingly, increased Erk activity via activation of CB2 receptors induces immune cell migration and changes in gene expression.[40,48] When expressed in AtT-20 cells, CB2 receptors do not modulate ion conductances, making this function unique to CB1 receptors.[27] This result poses an

intriguing question: considering that some neurons express CB2 receptors, does activation of this receptor affect the firing rate of these neurons and, if so, through what signal transduction pathway?

Two Endocannabinoids

The existence of cannabinoid receptors and the biological effects induced by cannabinoids suggests the existence of endocannabinoids that normally engage these receptors. Arachidonoylethanolamide (AEA), also known as anandamide, was identified in 1992 by Devane and colleagues and shown to bind with high affinity to CB1 receptors.[49] Since then, AEA has been extensively studied and shown to fulfill the 3 criteria necessary to be considered a bona fide endocannabinoid: (1) activity-dependent production, (2) functional activation of cannabinoid receptors, and (3) biological inactivation.

Activity-dependent production of AEA was demonstrated in neurons in primary culture and in the brain of freely moving rats using microdialysis.[50-52] The molecular mechanism underlying AEA biosynthesis and hydrolysis is beginning to be understood. Depending on the cell type, stimuli that increase intracellular concentrations of calcium increase AEA production.[50,53] Calcium-dependent increases in AEA production are likely mediated through calcium-dependent increases in the activity of acyltransferase, the enzyme that generates the AEA precursor *N*-arachidonylphosphatidylethanolamide (NAPE).[54,55] A recently cloned NAPE-phospholipase D may be responsible for cleaving this precursor.[56] Thus, unlike classical transmitter substances, AEA is produced upon demand by enzymatic cleavage of membrane lipid precursors and immediately extruded from cells without an intermediate step of vesicle storage, a feature reminiscent of other lipid-derived mediators.[57] AEA production is not restricted to neurons since many different cell types, including astrocytes and microglial cells, also produce this ligand.[40,58-62]

AEA activates the signal transduction pathways coupled to CB1 receptors, although it has a low intrinsic efficacy.[63] Injection of AEA into rodents mimics most of the effects produced by THC,[64] although inactivation of its degradation is often necessary to see biological effects.[65] AEA also binds CB2 receptors, acting as a partial agonist, or antagonist,[66,67] effects that might have some relevance in modulating inflammation.

Strong genetic and pharmacological evidence has demonstrated that fatty acid amide hydrolase (FAAH) inactivates AEA.[65,68] Of interest, other enzymes may also metabolize AEA, including cyclooxygenases and lipooxygenases.[69,70] Their respective involvement in AEA inactivation in intact cells, especially when FAAH is also expressed, is starting to be unraveled.[71,72]

In 1995, the laboratories of Raphael Mechoulam and Keizo Waku simultaneously reported a second endogenous ligand, 2-arachidonylglycerol (2-AG).[73,74] Neuronal activity enhances 2-AG synthesis, its levels reaching 100 times that of AEA.[75] While classic studies have shown that 2-AG is produced through the phospholipase C (PLC)/diacylglycerol lipase (DGL) pathway,[76,77] more recent genetic studies show that PLCβ1 and PLCβ4 mediate 2-AG biosynthesis in pyramidal and Purkinje neurons, respectively,[78,79] and that there exist 2 subtypes, α and β, of DGL directly controlling its biosynthesis.[80]

2-AG activates CB1 and CB2 receptors with distinct pharmacological profiles, is carried into cells by a "yet-to-be" cloned transporter, and is inactivated by monoacylglycerol lipase (MGL).[81-84] When MGL protein is fully knocked down by RNA interference (RNAi), 50% of the 2-AG hydrolyzing activity remains in cell homogenates, indicating that additional enzymes may hydrolyze this lipid.[85] Prime candidates are FAAH and the cyclooxygenases.[86,87]

In summary and as a first approximation, psychoactive effects of *cannabis* are primarily mediated by neuronal CB1 receptors, while immune effects are primarily mediated by CB2 receptors. Two endocannabinoids have been identified, their action on either CB1 or CB2 is thoroughly characterized, and their production and inactivation elucidated; however, many significant exceptions to these rules exist. Indeed, convincing evidence now suggests that specific cannabinoid effects on synaptic transmission, the cardiovascular system, and immune system are mediated by cannabinoid receptors distinct from CB1 and CB2, and that additional "independent" endocannabinoids exist. Below we will briefly review this evidence.

Non-CB1/CB2 Receptors on Neurons

Three sets of experiments, each using the approach of applying cannabinoids to $CB1^{-/-}$ mice, support the existence of non-CB1/CB2 receptors regulating synaptic transmission. The first were experiments examining GTPγS binding in brain membranes prepared from $CB1^{-/-}$ mice. Breivogel and colleagues found that AEA and the aminoalkylindole WIN55,212-2 stimulated GTPγS binding in brain homogenates prepared from these mice.[88,89] The regional distribution of GTPγS binding only partially overlapped those of CB1 or CB2 receptors, emphasizing that these novel receptors likely have a distinct physiological role. Furthermore, this novel receptor was stimulated by WIN55,212-2 and anandamide but not by CP55,940, HU210, or THC and was only weakly antagonized by SR141716A, emphasizing a pharmacology divergent from CB1 and CB2.

The second set of experiments supporting the existence of non-CB1/CB2 cannabinoid receptor(s) examined glutamatergic transmission in CA1 of mouse hippocampus.[90,91]

Hájos and colleagues found that WIN55,212-2 and CP55,940 inhibited excitatory transmission with equal efficacy in wild-type and $CB1^{-/-}$ mice. Of interest, a recent report showed that while CD1 mice had this response, the C57BL-6 strain did not.[92] This inhibitory effect of WIN55212-2 and CP55940 was abolished by SR141716 and capsazepine but was unaffected by the SR141716 analog, AM251.[90] As WIN55,212-2 and CP55,940 do not interact with TRPV1 channels, it is unlikely that TRPV1 mediates this response. Additional evidence suggested that these novel receptors are involved in endocannabinoid-mediated short-term plasticity. Specifically, Rouach and Nicoll[93] found that activation of group I metabotropic glutamate receptors in $CB1^{-/-}$ mice causes short-term depression of excitatory transmission in the hippocampal CA1 region that is blocked by SR141716. A pharmacologically similar receptor is also present on amygdala projection neurons, and this novel receptor likely plays a role in anxiety, as shown by antagonist experiments in rodents.[94]

The third set of experiments examined the analgesic efficacy of THC in spinal cord. Welch and coworkers found that the rank order potency of SR141716A in blocking analgesia produced by THC, anandamide, and CP55,490 differed from that predicted by a CB1-mediated response.[95,96] In addition, there were also differences in the synergy between morphine and THC compared with morphine and AEA or CP55,940, suggesting the involvement of a novel receptor.

Non-CB1/CB2 Receptors in the Vasculature

Very strong evidence for non-CB1/CB2 cannabinoid receptors comes from a continuing series of investigations performed by several laboratories studying the effects of cannabinoids on the vasculature.[97] Essentially the findings are that certain cannabinoids cause vasodilation and hypotension in the absence of CB1, CB2, or TRPV1 receptor activation. The best-characterized response is in mesenteric vessels,[98] in which AEA and its analog methanandamide cause vasodilation, while synthetic cannabinoids and THC do not. This response is sensitive to high concentrations (>1 μM) of SR141716A. Similar to the inhibitory effect on hippocampal glutamatergic transmission described above, the SR141716A analog AM251 does not antagonize this novel receptor. The response is, however, sensitive to pertussis toxin, implicating Gi or Go proteins in the signaling pathway. An important pharmacological tool for non-CB1/CB2 cannabinoid receptors that emerged from these studies is the cannabidiol analog, abnormal cannabidiol (abn-CBD).[98] Abn-CBD functions as an agonist at some of these novel cannabinoid receptors, is inactive at CB1 and CB2 receptors, and is antagonized by both cannabidiol and O-1918, a synthetic cannabidiol analog.[99] Several lines of evidence suggest that endothelial cells express this novel cannabinoid receptor, and its activation leads to the release of nitric oxide, culminating in the opening of potassium channels on vascular smooth muscle and leading to relaxation and vasodilation.[100] Although there are strong pharmacological parallels between the vascular and hippocampal novel receptors, there are some notable differences. For example, the vascular receptor is insensitive to potent synthetic cannabinoids. It is possible that such differences arise from distinct receptor entities, the specific cellular context in which the receptor is expressed or receptor dimerization.

Non-CB1/CB2 Receptors on Immune Cells

Immune cells express both CB1 and CB2 receptors, the levels of which vary depending on the activation state of immune cells. For example, while mature B cells express high levels of CB1 and CB2 receptors, naïve T cells express very low levels of either receptor.[101] Strong evidence suggests that cannabinoids interact with CB1 and CB2 receptors expressed by immune cells, increasing their proliferation rate and survival, while inhibiting the production of various immune mediators such as cytokines.[102,103] As such, many of the therapeutic, anti-inflammatory properties attributed to *C sativa* intake likely occur through this mechanism. Yet recent convincing evidence shows that immune cells likely express at least one additional cannabinoid receptor. These experiments were performed with palmitoylethanolamide (PEA, an analog of anandamide that contains a 16:0 fatty acid moiety instead of 20:4 for AEA), which has received considerable attention because of its antiinflammatory properties.[104] Piomelli and colleagues, and, simultaneously, Rice and colleagues, found that PEA reduces the pain associated with an inflammatory response.[58,105] This analgesic effect of PEA likely involves a novel cannabinoid receptor because SR144528, a well-characterized CB2 antagonist, blocked the analgesia, yet PEA does not bind to CB2 receptors.[66] Two hypotheses stem from these results: (1) PEA might interact with a novel cannabinoid receptor that is antagonized by SR144528 (thus this antagonist is not specific for CB2 receptors), or (2) PEA might stimulate a novel cannabinoid receptor that couples to phospholipases C and diacylglycerol lipase, increases 2-AG production, and thus indirectly activates CB2 receptors. Currently, the signal transduction pathway(s) coupled to the novel PEA-sensitive receptor is unknown.

PEA: An Independent Endocannabinoid?

Results showing that PEA interacts with a distinct non-CB1/CB2 receptor suggest that this lipid might constitute a unique "parallel" endocannabinoid signaling system. Providing support to this concept is the evidence that PEA

production and inactivation can occur independently of AEA and 2-AG production and inactivation. Specifically, in rodent cortical neurons, general activity-dependent production of AEA, 2-AG, and PEA occurs concomitantly.[50,106,107] Yet, subsequent studies showed that 2-AG production can be increased independently when N-methyl-D-aspartate (NMDA) receptors are activated, while increased AEA and PEA production requires addition of carbachol.[108] A more detailed pharmacological study showed that activation of nicotinic receptors increases AEA production, while activation of muscarinic receptors increases PEA production. Thus, although biosynthesis of all endocannabinoids in this model system is contingent on NMDA-receptor occupation, increased AEA production requires the co-activation of α7 nicotinic receptors, while increased PEA production requires the co-activation of muscarinic receptors. This finding suggests that glutamate and acetylcholine may elicit the biosynthesis of different endocannabinoids depending on the complement of cholinergic receptors expressed in their target neurons. Additional evidence for independent production of AEA, 2-AG, and PEA comes from experiments performed on mouse astrocytes in culture. In this model, the calcium ionophore ionomycin and the peptide endothelin-1 increase the production of both AEA and 2-AG, while PEA levels remain unchanged.[109,110] The notion that PEA might be independently produced also holds true in vivo.[111] For example, in the case of focal cerebral ischemia, PEA levels in ischemic cerebral cortex increase ~25-fold compared with sham-operated animals, while AEA levels increase by barely 3-fold and 2-AG levels remain unchanged.

Novel evidence shows that PEA inactivation can also occur independently from that of AEA and 2-AG. The laboratory of Natsuo Ueda discovered the existence of a unique enzyme capable of hydrolyzing PEA to much greater extent than AEA and 2-AG. The original observation was obtained with homogenates prepared from human megakaryoblastic cells (CMK), in which AEA hydrolysis occurred with low activity and a strikingly different pH profile from that of FAAH.[112] Specifically, while FAAH is known to maximally hydrolyze AEA at pH 9 (with this activity dropping by 70% at pH 5), CMK cells were shown to maximally hydrolyze AEA at pH 5 (with this activity dropping by 95% at pH 9). Using an elegant 4-step purification approach, Ueda and colleagues were able to purify this novel enzymatic activity by 760-fold, obtain partial protein sequence, and clone a cDNA encoding this protein, which was named N-acylethanolamine-hydrolyzing acid amidase (NAAA).[113] When assessing its substrate specificity, it became clear that this enzyme preferred PEA over AEA (having hydrolytic activities toward these substrates of 8 and 0.25 nmol/min/mg, respectively). Besides a distinct pH profile and substrate specificity, NAAA has additionally very interesting properties. It is highly expressed in spleen and thymus, as well as in macrophages homing to the lungs and small intestine, highlighting its potential importance in regulating PEA signaling in the context of immunobiology. Here, it should be emphasized that NAAA expression and activity are quite low in healthy brain.[113]

The laboratory of Didier Lambert developed a competitive inhibitor of NAAA, N-cyclohexanecarbonylpentadecylamine, which has an IC50 of 5 μM and is inactive against FAAH at 100 μM.[114] When considering that methyl arachidonoyl fluorophosphonate (MAFP) and URB597 inhibit FAAH with nanomolar IC50s and are both inactive against NAAA at a concentration of 1 μM, experiments designed to use these compounds in combination may be useful to distinguish the biological importance of either PEA or AEA hydrolysis in various biological responses.[114,115]

This series of studies raises many fascinating questions: for example, "What is the subcellular location of NAAA and does it differ from FAAH?" The pH profile of NAAA is quite intriguing. With maximal NAAA activity occurring at pH 5 and only 10% of this activity remaining at pH 7 (ie, the cytosolic pH), one wonders if NAAA might be active only in lysosomes. Accordingly, NAAA-GFP (green fluorescent protein) fusion protein localized to lysosome-like vesicles.[113] This result is quite interesting when considering that FAAH is also abundant in intracellular organelles such as mitochondria and the smooth endoplasmic reticulum.[116] Clearly, elucidating the exact biological role of NAAA will be facilitated by genetic studies similar to those performed on FAAH. Finally, while AEA and 2-AG hydrolysis give rise to new bioactive lipids (ie arachidonic acid and eicosanoids), PEA hydrolysis gives rise to 2 relatively inactive products, palmitic acid and ethanolamine, suggesting that the role of NAAA is to truly stop biological responses initiated by increases in PEA production.

Is GPR55 the Cannabinoid Receptor Engaged by PEA?

GPR55 was first identified as an orphan G protein coupled receptor (GPCR) enriched in brain.[117] Its gene is located on chromosome 2 (location: 2q37) in mice and chromosome 6 in humans, and its open reading frame encodes a relatively short 319 amino acid protein. Using Northern blot analysis of human tissues, GPR55 mRNA was found in caudate and putamen, but not in frontal cortex, hippocampus, thalamus, pons, cerebellum, or liver.[117] Northern blot analysis of rat tissues showed GPR55 mRNA in spleen, fetal tissues, and intestine. Further in situ hybridization studies found GPR55 mRNA in rat hippocampus, thalamus, and midbrain.[117] The difference between the human and rat CNS studies may represent sensitivity differences between the 2 specific techniques, or a variation between species, and invites further careful study. A more thorough distribution of human

GPR55 mRNA has been reported in the patent literature. A first patent reported the following relative abundance: adipose > testis > myometrium > adenoid = tonsil > spleen > ilium > brain = stomach.[118] Low levels were found in other tissues. Synthesis of the above results emphasizes that GPR55 is highly expressed in tissues known to respond to cannabinoids.

A very recent second patent application argued that GPR55 might constitute an additional cannabinoid receptor subtype.[119] The main findings are as follows: human GPR55 amplified from genomic DNA contains an 11 amino acid substitution in distal intracellular loop 2 and the fourth transmembrane domain not present in the originally reported GPR55 sequence. This variant was termed GPR55A and corresponds to the Human Genome Project sequence. Phylogenetically, GPR55 is closest in sequence to the platelet activating factor (PAF) purinergic P2Y9 and 2 orphan receptors GPR35 and GPR92. It has reasonable homology to several other very interesting GPCR, including P2Y5 and CCR4. It only shares 13.5% and 14.4% homology with CB1 and CB2, respectively. When expressed in HEK293 cells, GPR55A bound CP55,940 and SR141716A, but not WIN55,212-2. Signal transduction was examined using GTPγS binding. A wide range of cannabinoid compounds stimulated GTPγS binding, including THC, anandamide, 2-AG, virodhamine, and CP55,940, all with EC50 values less than 20 nM. Most remarkably, PEA also stimulated GTPγS binding with even lower nM potency. This latter result suggests the exciting possibility that GPR55 may mediate the antiinflammatory effects of PEA discussed above. Pretreatment of GPR55A expressing HEK293 membranes with pertussis toxin or cholera toxin did not alter CP55,940 stimulation of GTPγS binding, indicating that, in contrast to CB1 and CB2 receptors, GPR55A does not activate Gi, Go, or Gs proteins, at least in HEK293 cells. Thus, GPR55(A) represents a new subtype of cannabinoid receptor with ligand binding and signaling profiles distinct from those of CB1 and CB2.

Conclusion

The results discussed above clearly argue for the existence of multiple cannabinoid receptors, specifically the cloned CB1 and CB2 receptors, and at least 3 non-CB1/CB2 cannabinoid receptors. Owing to their expression profile and coupling mechanism, each receptor likely mediates distinct effects of phytocannabinoids and endocannabinoids. In addition, strong evidence implicates PEA as an inflammatory modulator acting via a non-CB1/CB2 cannabinoid receptor. While recent data suggest that this latter receptor might be GPR55, many key experiments remain to be done, such as determining the precise pharmacology of this receptor, its coupling capabilities and expression pattern. The existence of different enzymatic routes for the formation of AEA, 2-AG, and PEA suggests that these endocannabinoids may operate independently from each other.

While the place that cannabinoid receptors occupy in the field of pharmacology research is still evolving, strong evidence implicates their ability to regulate neuronal, vascular, and immune functions. An understanding of the expression, function, and regulation of these receptors, the molecular mechanism involved in the production and inactivation of their endogenous ligands, and how phytocannabinoids interfere with this signaling system is clearly important if we are rationally and comprehensively to assess the function of the cannabinoid signaling system in human health and disease.

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