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The cannabinoid receptors

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Abstract

Cannabinoid receptors were named because they have affinit for the agonist Δ^9-tetrahydrocannabinol (Δ^9-THC), a ligand found in organic extracts from *Cannabis sativa*. The two types of cannabinoid receptors, CB_1 and CB_2, are G protein coupled receptors that are coupled through the $G_{i/o}$ family of proteins to signal transduction mechanisms that include inhibition of adenylyl cyclase, activation of mitogen-activated protein kinase, regulation of calcium and potassium channels (CB_1 only), and other signal transduction pathways. A class of the eicosanoid ligands are relevant to lipid-mediated cellular signaling because they serve as endogenous agonists for cannabinoid receptors, and are thus referred to as endocannabinoids. Those compounds identified to date include the eicosanoids arachidonoylethanolamide (anandamide), 2-arachidonoylglycerol and 2-arachidonylglyceryl ether (noladin ether). Several excellent reviews on endocannabinoids and their synthesis, metabolism and function have appeared in recent years [1–4]. This paper will describe the biological activities, pharmacology, and signal transduction mechanisms for the cannabinoid receptors, with particular emphasis on the responses to the eicosanoid ligands.

Keywords: Cannabinoid receptors; Agonist; Presynaptic

1. Eicosanoids as cannabinoid receptor agonists

Endocannabinoids comprise a family of eicosanoid and related unsaturated fatty acid derivatives that stimulate cannabinoid receptors: arachidonoylethanolamide (anandamide) [5], homo-γ-linolenoylethanolamide, docosatetraenoylethanolamide [6], 2-arachidonoylglycerol [7,8] and 2-arachidonylglyceryl ether (noladin ether) [9]. Many analogs of anandamide have been developed such that a structure–activity relationship profil is beginning

to emerge, and some of these analogs are of experimental use (see recent reviews [4,10] for an overview). Of note, *R*-(+)-methanandamide, a synthetic analog of anandamide that is not readily biotransformed, exhibits somewhat greater affinit for CB_1 receptors than anandamide itself [11]. CB_1-selective agonists include arachidonyl-2′-chloroethylamide (ACEA), arachidonylcyclopropylamide (ACPA) [12] and O-1812 [13]. O-1812 exhibits higher affinit than anandamide for the CB_1 receptor and is not readily hydrolyzed [13].

2-Arachidonoylglycerol is somewhat selective for binding to CB_1 over CB_2 receptors [7,14]. The isomer 1(3)-arachidonoylglycerol has comparable binding affinit as 2-arachidonoylglycerol [3], and so caution must be given to adequate separation of isomers for biological determinations claiming activity for 2-arachidonoylglycerol. Other fatty acid substitutes, 2-palmitoylglycerol, 2-linoleoylglycerol, 1(3)-palmitoylglycerol and 1(3)-stearoylglycerol fail to bind to CB_1 or CB_2 receptors with reasonable affinit or mimic 2-arachidonoylglycerol's biological effects [3,7,14,15]. Noladin ether has much greater selective for CB_1 over CB_2 receptors [9]; however, its activity as a CB_1 agonist may be less than that exhibited by 2-arachidonoylglycerol [16].

2. Biological actions attributable to CB_1 receptors

Therapeutic applications for Δ^9-THC have been exploited in analgesia, attenuation of the nausea and vomiting in cancer chemotherapy, and appetite stimulation in wasting syndromes (see Pertwee [17,18] and Porter and Felder [19] for reviews). However, the pharmaceutical industry has hesitated to promote these agents due to the untoward side effects of alterations in cognition and memory, dysphoria/euphoria, and sedation (see Abood and Martin [20], Ameri [21], and Chaperone and Thiébot [22] for review). Cannabinoid drugs have been evaluated in humans in controlled tests for subjective perceptions of "high" as well as objective measures such as tachycardia [23–25], and the CB_1-selective antagonist SR141716 was able to block both responses [26]. Observations of overt behavior in monkeys describe a pattern of sedation, ptosis, and body sag in response to cannabinoid drugs [27]. In cynomolgus monkeys, cannabinoid agonists decreased general and locomotor activity, and increased bradykinesia, but did not induce freezing or catalepsy [28]. Cannabinoid drugs impaired learning and memory in nonhuman primates [29], as it does in humans [30].

An excellent correlation exists between the cannabinoid subjective effects in humans and drug discrimination in nonhuman primates and rodents [31]. The eicosanoid ligand anandamide has been predicted to produce cannabinoid behavioral effects in humans based upon drug discrimination studies [32,33]. SR141716 was shown to block the discriminative properties of Δ^9-THC [34,35], thereby implicating CB_1-mediated effects as determining criteria.

In rodents, cannabinoid drugs produce a "tetrad" of characteristic pharmacological effects: antinociception, hypothermia, a decrease in general mobility (sedation), and catalepsy, the combination of which has achieved acceptability as a screening procedure [36]. Structure–activity relationship determinants in the mouse tetrad model have been reported for eicosanoid ligands, including noladin ether [9,37]. The selective CB_1 antagonist SR141716 was effective in blocking the effects of most cannabinoid drugs in the mouse tetrad model [38,39]; however, some discrepancies have been noted with anandamide [37]

(see further discussion below). Cannabinoid drugs impaired learning and memory in rodents in the delayed match-to-sample task [40] and the eight-arm radial maze [41,42], and these effects were blocked by SR141716 [42]. SR141716 given alone was able to improve memory in rats [43], suggesting a role for endocannabinoids in neuronal functions associated with memory.

Consistent with the behavioral expectations, cannabinoid receptors have been found in sensory and autonomic nervous systems, and in the central nervous system where they are abundant in cerebral cortex, hippocampus, basal ganglia, and cerebellum, less abundant in hypothalamus and spinal cord, and very sparse in the brainstem (see Elphick and Egertová [44] and Howlett et al. [10] for review and references). CB_1 receptors were found on axons and axon terminals according to immunocytochemical studies [45,46]. Electron microscopic studies demonstrated that CB_1 receptors were found abundantly on presynaptic terminals [47–51]; but they were also found on postsynaptic structures and glia [52].

Agonist stimulation of presynaptic CB_1 receptors has been demonstrated to inhibit release of a number of excitatory or inhibitory neurotransmitters, both in the brain and in the peripheral nervous system (see Howlett et al. [10] and Schlicker and Kathman [53] for review). This leads to the question of whether the eicosanoid ligands anandamide and 2-arachidonoylglycerol fulfil the criteria to be classifie as neurotransmitters or neuromodulators. For these two endocannabinoids (but not noladin ether), evidence exists to support:

1. synthesis and release from neurons in response to neurotransmitters or depolarization and Ca^{2+} [54–59];
2. endocannabinoid mimicry of the response to a neuronal stimulus (see Di Marzo et al. [60] for review);
3. rapid removal from the extracellular space by a membrane transport process [1,60–63]; and
4. biotransformation via a microsomal enzyme, fatty acid amide hydrolase (FAAH) [1,60,62,64,65]. It has been speculated that CB_1 receptors on presynaptic neurons and FAAH on postsynaptic neurons can be paired as a mechanism of synaptic interaction [46,66].

Observations such as these leave us to speculate that the endocannabinoids might serve as retrograde synaptic messengers in controlling neurotransmitter release.

Anandamide and the metabolically stable analog *R*-(+)-methanandamide stimulated [^{35}S]GTPγS binding to G proteins in rat cerebellar membranes in which phenylmethylsulfonyl fluorid had been used to inhibit membrane FAAH activity [12,67–69]. In those studies, the maximal stimulation was 60–80% of that of cannabinoid or aminoalkylindole full agonists, thereby characterizing anandamide as a partial agonist in this activity. 2-Arachidonoylglycerol exhibited low potency in rat brain membranes but had the same effica y as a full agonist [70].

Anandamide, *R*-(+)-methanandamide, and 2-arachidonoylglycerol were full agonists to inhibit forskolin-stimulated cAMP synthesis in mouse N18TG2 neuroblastoma cells or membranes [71,72]. Anandamide inhibited adenylyl cyclase activity in rat cerebellar membranes with a maximal inhibition of about 80% of that reported for full agonists [73]. Anandamide also attenuated cAMP accumulation in intact CHO cells expressing

recombinant CB_1 receptors [12,71,74]. Thus, anandamide acts as an agonist with low potency but high effica y, whereas 2-arachidonoylglycerol behaves with higher potency in the cAMP signal transduction pathway.

Anandamide inhibited N-type voltage-gated Ca^{2+} channels in differentiated N18 neuroblastoma cells via the CB_1 receptor and a $G_{i/o}$ protein [74,75]. Anandamide behaved as a relatively high potency partial agonist, and as such could antagonize the response to the full agonist WIN55212-2 [75]. The response was mimicked by arachidonyl side chain analogs dihomo-γ-linolenyl, adrenyl, and mead ethanolamide derivatives [74,76]. 2-Arachidonoylglycerol inhibited the depolarization-evoked rise in intracellular Ca^{2+} in differentiated NG108-15 cells [77]. This effect was mimicked by 2-linolenylglycerol and 2-docosahexaenoylglycerol [77]. Anandamide was a partial agonist in this response, and arachidonic acid was without effect. Anandamide was a full agonist to inhibit Q-type Ca^{2+} currents in AtT-20 pituitary tumor cells expressing recombinant CB_1 receptors and endogenous $G_{i/o}$ proteins [78]. However, anandamide was not able to inhibit Q-type Ca^{2+} currents in the same host cells expressing recombinant CB_2 receptors, indicating that the CB_2 receptor fails to couple to this channel [79]. In rat cortical and cerebellar brain slices, anandamide inhibited P/Q-type Ca^{2+} flu es via the CB_1 receptor and $G_{i/o}$ proteins [80]. In arterial smooth muscle cells, anandamide evoked a concentration-dependent inhibition of L-type Ca^{2+} currents, which could be blocked by pertussis toxin and by SR141716, suggesting that regulation was via the CB_1 receptor [81].

Anandamide was a full agonist to activate the inwardly rectifying K_{ir} channels in AtT-20 cells expressing exogenous CB_1 receptors and endogenous $G_{i/o}$ [78]. When tested in *Xenopus laevis* oocytes coexpressing the CB_1 receptor plus GIRK1 and GIRK4 channels, anandamide exhibited a maximal response that was 50% of that observed for a full agonist [82].

Mitogen-activated protein kinase (MAPK) pathways were shown to be activated via the CB_1 receptor in cultured astrocytic cells and expression systems [83]. The MAPK activation was linked to expression of the immediate early gene product Krox-24 in human astrocytoma cells [84]. Intracerebroventricular injection of anandamide evoked an increase in immediate early gene product cFos immunoreactive protein in rat brain, and this response followed a generally similar distribution to that of CB_1 receptors, suggesting a correlation [85]. MAPK activation led to activation of the Na^+/H^+ exchanger in cannabinoid-stimulated CHO cells stably expressing recombinant CB_1 receptors [86]. In WI-38 fibroblasts anandamide promoted *tyr*-phosphorylation of Erk2 and increased MAPK activity, and this was accompanied by phosphorylation of cytoplasmic phospholipase A_2, [^{3}H] arachidonic acid release, and prostaglandin E_2 synthesis [87]. Anandamide stimulated *tyr*-phosphorylation of pp125-focal adhesion kinase (Fak), and this response was mediated by the CB_1 receptor via the inhibition of adenylyl cyclase [88].

In C6 glioma and primary astrocyte cultures, CB_1 agonists increased glucose metabolism, phospholipid synthesis and glycogen synthesis via a pertussis toxin-sensitive mechanism [89,90]. Two signal transduction pathways were proposed: (1) activation of phosphatidylinositol-3 (PI3)-kinase, mediating *tyr*-phosphorylation and activation of Raf-1, leading to MAPK phosphorylation and ultimately glucose oxidation; (2) sphingomyelin hydrolysis, release of ceramide, and the subsequent activation of the Raf-1/MAPK cascade. In the latter pathway, ceramide could also activate carnitine palmitoyltransferase on mitochondrial membranes to stimulate ketogenesis and fatty acid oxidation.

Both endocannabinoids anandamide and 2-arachidonoylglycerol evoked a rapid, transient increase in intracellular free Ca^{2+} in undifferentiated neuroblastoma and neuroblastoma-glioma hybrid cells, and this is believed to be mediated by the CB_1 receptor and $G_{i/o}$ proteins [91–93]. Arachidonic acid and 2-palmitoylglycerol, lipids that fail to interact with the CB_1 receptor, failed to evoke the response [92]. Classical and nonclassical cannabinoid ligands as well as anandamide and *R*-(+)-methanandamide produced much lower maximal responses than did 2-arachidonoylglycerol, 1(3)-arachidonoylglycerol or 2-hydroxypropylarachidonate [91–93]. This relative effica y profil differs from that described for [^{35}S]GTPγS binding and inhibition of adenylyl cyclase, suggesting that the signal transduction pathway for this intracellular Ca^{2+} regulation must differ, perhaps in the type of G protein that transduces the response. A phospholipase C inhibitor blocked the response, suggesting that an increase inositol-1,4,5-triphosphate (IP_3) might mediate a release of Ca^{2+} from intracellular stores [91]. However, in CHO cells expressing recombinant CB_1 or CB_2 receptors, cannabinoid drugs and anandamide failed to elicit changes in IP_3 or phosphatidic acid under conditions in which stimulation of recombinant muscarinic receptors was effective [74,79]. Thus, CB_1 receptor-mediated activation of phopholipase C is not a commonly observed signal transduction pathway.

Anandamide elicited nitric oxide (NO) release in human monocytes [94], rat median eminence fragments [95], human saphenous vein segments [96], and cultured human endothelial cells [97,98]. Antagonism by SR141716 suggested that this response was mediated by CB_1 receptors, and antagonism by (L)-*N*-*arg*-methyl ester (L-NAME) implicated an isoform of nitric oxide synthase (NOS). In cultured human endothelial cells, NO generation was preceded by a rapid increase in intracellular Ca^{2+} concentration [97,98], consistent with the stimulation of a Ca^{2+}-regulated constitutive NOS. In saphenous vein endothelia, an influ from the extracellular Ca^{2+} pool was required for NOS activation [96].

3. Biological actions attributable to CB_2 receptors

In situ hybridization, immunocytochemical and autoradiographic evidence demonstrates the presence of CB_2 receptors in spleen, thymus, tonsils, bone marrow, pancreas, splenic macrophage/monocyte preparations, mast cells, peripheral blood leukocytes, and in a variety of cultured immune cell models, including the myeloid cell line U937 and undifferentiated and differentiated granulocyte-like or macrophage-like HL60 cells (see recent reviews by Berdyshev [99] and Cabral [100]). Cannabinoid drugs modulated functional activities of macrophages and macrophage-like cells to process antigens required for the activation of T lymphocytes [101,102]. Mediation by CB_2 receptors was shown in experiments in which Δ^9-THC inhibited helper T cell activation through macrophages derived from wild type, but not from CB_2 receptor knockout mice [103]. The formyl-methionyl-leucine-phenylalanine (fMLP)-induced chemotaxis was attributable to CB_2 receptors [104]. However, some immune effects are attributable to both CB_1 and CB_2 receptors, such as the decreased spontaneous migration of macrophages in vitro [104]. Both CB_1 and CB_2 cannabinoid receptor types take part in Δ^9-THC-induced inhibition of natural killer cell activity [105]. It has been hypothesized that Δ^9-THC augments the expression of immune inhibitory Th2-type cytokines, and inhibits expression of Th1-type immune stimulatory cytokines [100]. Δ^9-THC

inhibited antitumor immunity by a CB_2 receptor-mediated, cytokine-dependent pathway [106]. Δ^9-THC treatment of BALB/c mice suppressed immunity and early interferon-γ, interleukin-12, and IL-12 receptor β2 responses to *Legionella pneumophila*, which are mediated by both the CB_1 and CB_2 receptors [107].

Signal transduction initiated by the CB_2 receptor is mediated via $G_{i/o}$ proteins. 2-Arachidonoylglycerol was a full agonist, whereas anandamide was a partial agonist in the stimulation of [^{35}S]GTPγS binding to G proteins in CHO cells expressing recombinanat CB_2 receptors [12,108]. 2-Arachidonoylglycerol and anandamide both behaved as full agonists to stimulate [^{35}S]GTPγS binding to G proteins in membranes from Sf9 insect cells expressing recombinant human CB_2 receptors [108].

In CHO cells expressing CB_2 receptors, anandamide had mixed effects regarding its ability to inhibit forskolin-stimulated cAMP accumulation [12,79,108,110]. These studies suggest that anandamide is a low potency (and possibly low effica y) agonist on CB_2 receptors compared with CB_1 receptors in the same preparation. 2-Arachidonoylglycerol was a low potency full agonist to inhibit forskolin-stimulated cAMP accumulation in CHO cells expressing recombinant CB_2 receptors [108]. In human HL-60 promyelocytic leukemia cells that express the CB_2 receptor, 2-arachidonoylglycerol induced a rapid transient increase in intracellular free Ca^{2+} concentrations, which was blocked by the CB_2 antagonist, SR144528 [109]. Anandamide was a weak partial agonist and palmitoylethanolamide was inactive. MAPK pathways were activated via the CB_2 receptor in cultured human promyelocytic HL60 cells and expression systems [111]. CB_2 receptor-mediated MAPK activation could be linked to expression of immediate early gene product Krox-24 in HL60 promyelocytes [111].

4. Speculation regarding alternative receptors for eicosanoid ligands

Eicosanoid drugs have been shown to stimulate activities beyond those expected from CB_1 receptor activation alone. SR141716 failed to block the effects of anandamide in the tetrad model for mouse behaviors [37]. Oddly, it was capable of blocking the effects of several anandamide analogs that had been developed for their metabolic stability [37]. Furthermore, some anandamide analogs were effective in the mouse tetrad model, but exhibited low affin ity for CB_1 receptors [13]. One explanation for these discrepancies is that other receptor type(s) might also mediate eicosanoid effects. One means to identify alternative receptors for eicosanoid ligands is to examine responses in cannabinoid receptor-deficien animals. Transgenic mice bearing a genetic deletion of the CB_1 or CB_2 receptors as well as CB_1/CB_2 double knockouts have been created in either C57Bl/6J or CD1 strains of mice [103,112, 113]. Anandamide elicited antinociceptive effects and a decrease in locomotor activity in CB_1 receptor knockout mice resembling the effects observed in wild-type mice, and SR141716 was not able to block the response [114]. This findin suggests either that an alternative receptor for anandamide exists in the brain, or that the effects of a metabolite of anandamide are being observed. Signal transduction studies indicated that both anandamide and WIN55212-2, but not classical or nonclassical cannabinoid agonists, could stimulate [^{35}S]GTPγS binding in CB_1 knockout mice, indicating that pharmacological characterization could be used to distinguish these novel receptors [115]. [^{35}S]GTPγS binding was

stimulated in brain membranes from CB_1 knockout mice by μM concentrations of anandamide or WIN55212-2 but not by Δ^9-THC or other classical or nonclassical cannabinoid ligands. SR141716 had no ability to antagonize the response. Cerebral cortex, hippocampus and brain stem membranes from CB_1 knockout mice were found to contain specifi binding sites for [^{3}H]WIN55212 but not [^{3}H]CP55940, and regional differences in binding densities distinguished this binding site from the CB_1 receptor [115].

In a second example of a non-CB_1 receptor-mediated endocannabinoid response, anandamide stimulated vasodilation of precontracted mesenteric arterial bed [116,117], and a similar response was observed in rabbit aortic rings [118]. However, the pharmacological profil appears to be quite different from that described for the novel anandamide/WIN55212-2 receptor in the CNS [116]. The pharmacological characterization demonstrated that anandamide and *R*-(+)-methanandamide could elicit vasodilation, but 2-arachidonoylglycerol, classical or nonclassical cannabinoids and aminoalkylindole ligands could not [116–118]. The response could be antagonized by SR141716, and thus, the receptor has been characterized as "CB_1-like". Other agonists for this novel receptor include "abnormal cannabidiol" and O-1602, which exhibit limited affinit for CB_1 receptors [116]. Anandamide, *R*-(+)-methanandamide, and abnormal cannabidiol also relaxed (attenuated by SR141716 and cannabidiol) mesenteric arteries obtained from CB_1 receptor knockout mice or from CB_1/CB_2 double-knockout mice, confirmin that this response is due to a novel receptor [116]. Evidence obtained from endothelium-denuded preparations suggested that this novel anandamide-sensitive, SR141716-blockable receptor was localized to endothelial cells [117–119].

A third non-CB_1/non-CB_2 receptor-mediated response appeared when it was reported that local injection of palmitoylethanolamide induced an antinociceptive response in the mouse formalin paw test as well as in the mouse abdominal stretch test, and this response could be attenuated by the CB_2 antagonist, SR144528, but not the CB_1 antagonist, SR141716 [120,121]. It was also reported that the antinociceptive response to anandamide could be antagonized by SR141716 but not SR144528, and that palmitoylethanolamide and anandamide acted synergistically [120,121]. The mechanisms for the antinociceptvie response would be unlike that of anandamide because palmitoylethanolamide did not elicit an antinociceptive response in the mouse hot plate test [121]. Lipopolysaccharide-induction of inducible NOS in murine RAW264.7 macrophage-like cells could be inhibited by palmitoylethanolamide, but the pharmacology must be complex because cannabinoid drugs could also produce this effect [122]. Because palmitoylethanolamide exhibits poor affinit for CB_1 or CB_2 receptors [5,74,123–125], a novel receptor was proposed to explain the SR144528-sensitive, non-CB_2 response [121]. Evidence in support of a CB_2-like receptor in mouse vas deferens has also been reported [126].

5. Summary

Two mammalian cannabinoid receptors, CB_1 and CB_2, have been pharmacologically characterized and anatomically localized. Endogenous lipid mediators of the eicosanoid class, notably arachidonoylethanolamide (anandamide), 2-arachidonoylglycerol and 2-arachidonylglyceryl ether (noladin ether), bind to both cannabinoid receptor types and

evoke responses. CB_1 receptors are found predominantly in the central and peripheral nervous system, where they have been implicated in presynaptic inhibition of transmitter release. CB_2 receptors are present on immune cells, where they may be involved in cytokine release. Studies of CB_1, CB_2, and CB_1/CB_2 knockout mice have provided pharmacological evidence for additional types receptors that respond to the eicosanoid ligands; however, evidence of genetic sequences having significan homology to the currently cloned cannabinoid receptors is not available at the present time. The existence of novel receptors responding to the endocannabinoid class of eicosanoids may be of great significanc in definin the biological properties of these eicosanoid ligands, and may lead to the development of novel agonist ligands having pharmacologically selective properties.

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