Interactions of Cannabinoids With Biochemical Substrates

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ABSTRACT: Recent decades have seen much progress in the identification and characterization of cannabinoid receptors and the elucidation of the mechanisms by which derivatives of the *Cannabis sativa* plant bind to receptors and produce their physiological and psychological effects. The information generated in this process has enabled better understanding of the fundamental physiological and psychological processes controlled by the central and peripheral nervous systems and has fostered the development of natural and synthetic cannabinoids as therapeutic agents. A negative aspect of this decades-long effort is the proliferation of clandestinely synthesized analogs as recreational street drugs with dangerous effects. Currently, the interactions of cannabinoids with their biochemical substrates are extensively but inadequately understood, and the clinical application of derived and synthetic receptor ligands remains quite limited. The wide anatomical distribution and functional complexity of the cannabinoid system continue to indicate potential for both therapeutic and side effects, which offers challenges and opportunities for medicinal chemists involved in drug discovery and development.

KEYWORDS: Cannabinoids, endocannabinoids, cannabinoid receptor subtypes, synthetic cannabinoids, cannabinoid ligands, Δ⁹-THC

Introduction

Cannabis sativa is one of society's oldest cultivated plants, with records of its propagation and use dating back thousands of years. Due to widespread consumption for medicinal and recreational purposes, the manner in which the chemical constituents in cannabis produce their pharmacological effects has been the subject of considerable interest and investigation. Initially, the production of crude but concentrated forms, such as hashish and tinctures, showed that the pharmacologically active chemical constituents could be isolated and studied. As a result, the molecular structures of the unique compounds in cannabis (the phytocannabinoids) and their associated pharmacological activities were further defined. These efforts culminated in the structural elucidation of the principal psychoactive constituent, Δ⁹-THC (Figure 1), and facilitated the discovery of extremely potent synthetic cannabimimetic compounds, identification of their specific receptors and mechanisms of action, and further elucidation of the underlying biochemical systems through which cannabinoids exert their pharmacological actions. This article is an overview of the current understanding of the biomolecular basis for the pharmacological effects of cannabis and Δ⁹-THC–like cannabimimetics in humans. Emphasis is on (1) the types of cannabinoid receptors and their anatomical distribution; (2) the endogenous lipid signaling molecules that interact with these receptors (the endocannabinoids); (3) the enzymes and cellular processes involved in the synthesis, liberation, and degradation of the primary endocannabinoids *N*-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol; and (4) the role of the endocannabinoid system in the modulation of cellular signaling processes and neuronal excitability in areas of the brain effecting central nervous system function and behavior.

Cannabinoid Receptors

Although interest in the pharmacology of cannabis dates back several millennia, unequivocal evidence supporting the existence of specific cannabinoid receptors in the central nervous system (CNS) of animals began to accumulate only recently. In the 1980s, extremely potent synthetic compounds (Figure 2) with cannabimimetic activity in laboratory animals were shown to have rigorous structure-activity relationships, including stereoselectivity.[1–4] Further indication of the interaction of cannabinoids with specific receptor proteins was revealed through their ability to dose-dependently inhibit adenylate cyclase in a pertussis toxin–sensitive fashion that correlated with their pharmacological potency in laboratory animals.[5–7] Radiolabeling of the potent synthetic cannabinoid CP-55,940 provided proof of a saturable, high-affinity stereoselective biomolecular site in rat brain whose binding affinity for cannabinoids correlated to both the inhibition of adenylate cyclase in vitro and analgesic activity in vivo.[8] Autoradiography studies subsequently revealed the anatomical distribution of these high-affinity binding sites in the CNS[9–13] and peripheral tissues.[14] The pattern and density of distribution of the binding sites for cannabinoids in the brains of rats, dogs, monkeys, and humans were exceptional, with extremely high concentrations in the basal ganglia, hippocampus, cerebral cortex, and cerebellum (Figure 3). The widespread distribution of binding sites in

the CNS correlates with the diverse effects of cannabinoids observed in laboratory animals and humans, including alterations in movement (eg, locomotor activity, coordination, and catalepsy), feeding and satiety ("the munchies"), learning, memory, and affective states. The unique pattern of distribution facilitated the cloning, sequencing, and identification of a 7-transmembrane-spanning G protein–coupled cannabinoid receptor (GPCR) in the brain (CB_1) that was shown to be responsible for many of the diverse pharmacological actions of cannabinoids.[15–17] A second receptor (CB_2) was subsequently cloned and sequenced and found to be expressed in macrophages in the marginal zone of spleen and in other cells with immunological function.[18] While other receptors are substrates for certain cannabinoids, CB_1 and CB_2 are most closely associated with the pharmacological actions of Δ^9-THC and other psychoactive cannabinoids in humans.

The CB_1 and CB_2 subtypes couple preferentially to G proteins of the G_i and G_o classes.[7,19–21] The GPCR signal transduction pathway serves to amplify intracellular processes in response to the formation of the ligand-bound receptor complex. On binding of a cannabinoid ligand, the conformational equilibrium of the GPCR is altered. A cannabinoid agonist alters the receptor conformations into those that activate the

Figure 1. Δ^9-THC, the principal psychoactive constituent in *Cannabis sativa.*

trimeric G protein, causing G_α to bind a molecule of guanine triphosphate (GTP) and dissociate from the other two G protein subunits. The activated G protein subunits disengage from the receptor and modulate signal amplification pathways involving several downstream effector proteins, including inhibition of specific adenylate cyclases and voltage-dependent calcium channels, and activation of inwardly rectifying potassium channels and several mitogen-activated protein kinases (with some variation depending on cell type, levels of receptor expression, and endogenous tone or constitutive activity; see Figure 4). Cannabinoid ligands can vary in their intrinsic activity or efficacy in producing conformational changes that result in a subsequent receptor-mediated response, with full agonists producing a maximal cellular response, partial agonists an intermediate response, antagonists no response (but reducing or blocking the effect of another ligand), and inverse agonists decreasing the constitutive activity of the receptor signaling complex, that is, driving the basal level of responsiveness negative, which is opposite to the effect of agonists. For example, Δ^9-THC is a partial agonist with respect to its ability to stimulate GTP binding through the CB_1 receptor compared with CP-55,940, which is a full agonist.[22–24] This may help explain why smoking "Spice" and other synthetic cannabinoid–containing herbal formulations causes more severe panic attacks and other adverse effects than smoking cannabis.

The discovery of the CB_1-selective receptor inverse agonist SR141716A (rimonabant) (Figure 5)[26,27] permitted the effects of Δ^9-THC and opposing endogenous cannabinoid function to be further illuminated. For example, this compound was shown to produce physical withdrawal signs in rats made tolerant to the effects of Δ^9-THC.[28] Many of the central effects produced by Δ^9-THC, such as increased food consumption and impaired learning and memory, were attenuated by SR141716A,[29,30] providing further evidence of mediation through CB_1.
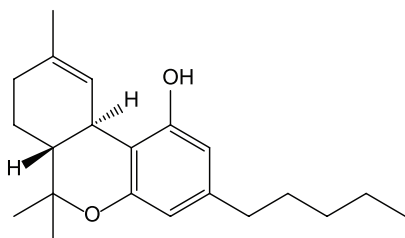
Levonantradol
(130-fold more potent than Δ^9-THC)

CP-47,497
(9-fold more potent than Δ^9-THC)

CP-55,244
(455-fold more potent than Δ^9-THC)

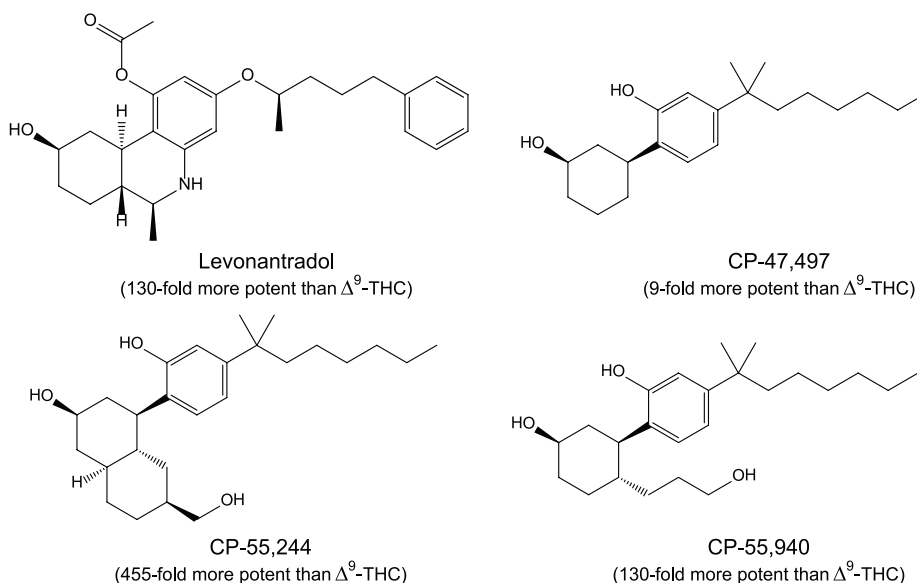**CP-55,940**
(130-fold more potent than Δ^9-THC)

Figure 2. Four potent synthetic cannabinoids.

Furthermore, treatment with SR141716A alone enhanced spatial memory,[31] decreased food consumption,[32] and produced other pharmacological effects indicative of its ability to act as an inverse agonist or reverse existing endocannabinoid tone at the CB_1 receptor. SR141716A also blocked the discriminative stimulus effects of Δ^9-THC in rodents and reduced the reinforcing effects and reinstatement of palatable food[33–35] and other drugs of abuse in animal self-administration models,

including heroin,[36] cocaine,[37] nicotine,[34,38,39] and ethanol.[33,40] The CB_2-selective inverse agonist SR144528 (Figure 5) was identified by the same research group at Sanofi[41] and shown to be a useful probe for cannabinoid-mediated effects in immune function.[42]

Cannabinoid receptors also contain allosteric sites that bind small molecules that alter receptor structure and function—that is, allosteric modulators. Allosteric sites are topographically distinct from those bound by orthosteric agonists (eg, Δ^9-THC and CP-55,940) and antagonists (eg, SR141716A and SR144528). When allosteric modulators bind to the allosteric site, they alter the conformational equilibrium of the receptor, which affects the ability of the receptor to bind compounds at the orthosteric site and couple to signal transduction molecules. Thus, allosteric modulators may either positively or negatively modulate the affinity or efficacy of orthosteric agonists and antagonists. Both positive and negative allosteric modulators (Figure 6) of cannabinoid receptors produce pharmacological effects distinct from those produced by orthosteric ligands.[43–51] Theoretically, allosteric modulators possess no basal activity in the absence of orthosteric ligands. Because of this, allosteric modulators might provide more selective spatial and temporal signaling,[52] exerting their effects only in the
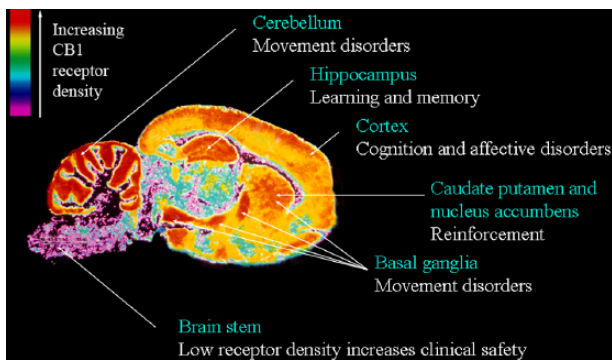

Figure 3. Radioligand autoradiograph of specific binding of [³H] CP-55,940 to a sagittal section of rat brain, showing high CB_1 receptor densities in the hippocampus, basal ganglia, and cerebellum.
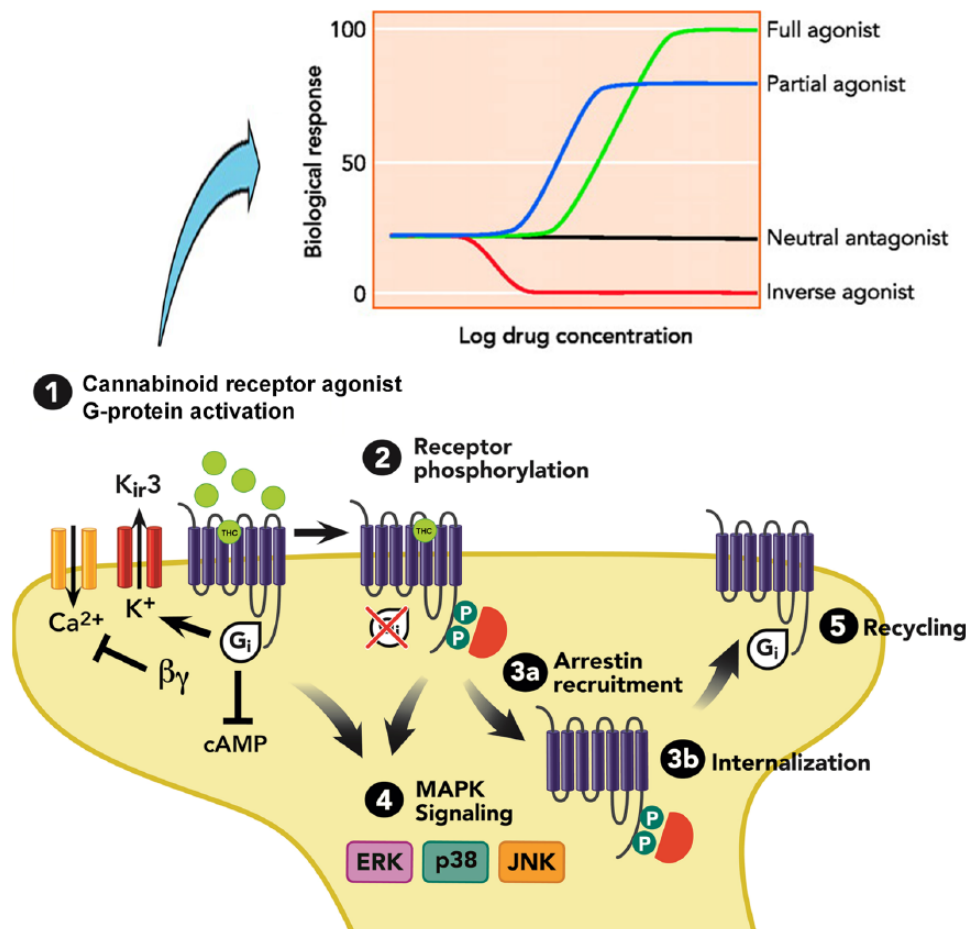

Figure 4. Cannabinoid receptor signaling and trafficking. Modified and reproduced with permission from Al-Hasani and Bruchas.[25]

presence of orthosteric ligands such as endocannabinoids, which are transiently released on demand and removed from their sites of action by enzymatic hydrolysis or cellular uptake. Thus, this fine-tuning of endogenous ligand signaling through orthosteric binding sites, instead of directly activating or blocking the receptor, may provide a promising alternative strategy for treating cannabinoid receptor–mediated disorders. However, as some compounds might bind to the allosteric site and act alone to alter the receptor's inherent coupling to signaling molecules (constitutive activity and signaling bias), it is also possible for compounds to act as allosteric agonists and allosteric inverse agonists, exerting their effects in the absence of an orthosteric ligand.[53,54] Interestingly, there are phytocannabinoids in cannabis,[47] as well as endogenous chemical constituents found in humans,[49,55,56] that have been reported to produce allosteric modulation of cannabinoid receptor function.

Endogenous Cannabinoid Signaling Molecules (Endocannabinoids)

The sequencing and cloning of the cannabinoid receptors and the availability of high-affinity radiolabeled ligands facilitated the screening and identification of endocannabinoids that act as cannabinoid receptor modulators. The first endocannabinoid, anandamide (Figure 7), was isolated and identified in the porcine brain.[57,58] It was shown to have an affinity (K_i) of approximately 50 nM for the CB_1 receptor[57] and a much lower affinity of 1600 nM for CB_2.[19] Another endocannabinoid lipid, 2-arachidonoylglycerol (2-AG), was isolated and identified in canine gut and shown to bind to both CB_1 and CB_2 with K_i values of approximately 500 and 1400 nM, respectively.[59] Like Δ^9-THC,
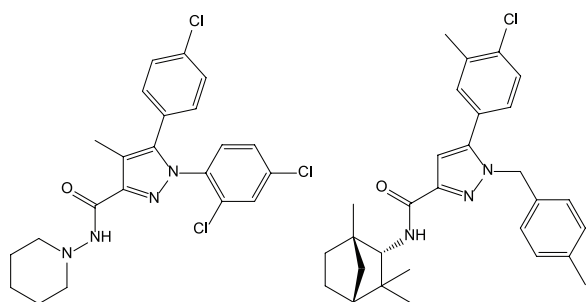
both compounds inhibit adenylate cyclase in isolated mouse spleen cells and electrically evoked contractions of the mouse vas deferens and produce the typical tetrad of pharmacological effects after intravenous administration in mice: antinociception, immobility, reduction in spontaneous activity, and reduction in rectal temperature.[57,59–61] 2-Arachidonoylglycerol is generally considered to be an orthosteric full agonist and anandamide an orthosteric partial agonist at both cannabinoid receptor subtypes in most tissues and signal transduction assays.[62,63] Many other endogenous and synthetic lipids have since been shown to interact with cannabinoid receptors.

Anandamide is derived from the precursor *N*-arachidonoyl phosphatidylethanolamine, which is synthesized by the relatively nonselective enzyme *N*-acyltransferase.[64,65] In the brain, anandamide and other *N*-acylethanolamines are released "on demand" in an activity-dependent manner by enzymatic cleavage of lipid precursors by phospholipase D[66] and other distinct biochemical pathways.[67–69] When released from the membrane, these lipids are typically bound reversibly to carrier or transport proteins called fatty acid–binding proteins.[70] *N*-acylethanolamines distributed in this way can act as diverse cellular signaling molecules in neural and nonneural tissues. Anandamide and other fatty acid amides are primarily degraded through the actions of fatty acid amide hydrolases (FAAHs).[71–73] Inhibitors of FAAH activity lead to increased levels of anandamide and other fatty acid amides and prostamides in tissues and biological fluids. Anandamide is also a substrate for metabolism through cyclooxygenase 2 (COX-2), resulting in the production of bioactive prostamides. Inhibition of FAAH and/or COX-2 increases endocannabinoid tone and is recognized as having therapeutic potential distinct from that available with exogenous receptor agonists of cannabinoid receptors.[74] FAAH (–/–) knockout mice have elevated levels of anandamide in the brain and demonstrate analgesia that is reversed by the CB_1 antagonist SR141716A.[75] Moreover, several FAAH inhibitors have analgesic and anti-inflammatory activity in animal models[76,77] and have been tested in clinical trials for a variety of therapeutic end points.[78,79] However, there is heightened awareness of the risks for significant adverse events with this relatively indirect approach to cannabinoid therapeutics after a fatality, and severe neurologic disorders were encountered in clinical trials of the FAAH inhibitor BIA 10-2474.[80] Although this compound is suspected to have a prolonged and broad

Figure 5. The CB_1- and the CB_2-selective inverse agonists SR141716A (left) and SR144528 (right).
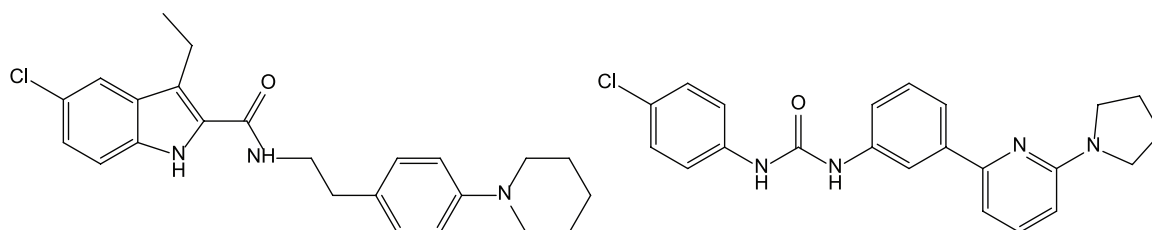

Figure 6. CB_1 allosteric modulators. Org 27569 (left) and PSNCBAM-1 (right) display positive binding cooperativity with the orthosteric agonist CP-55,940 for CB_1 receptor binding as would positive allosteric modulators, but reduce the efficacy of orthosteric agonists in several in vitro functional assays.
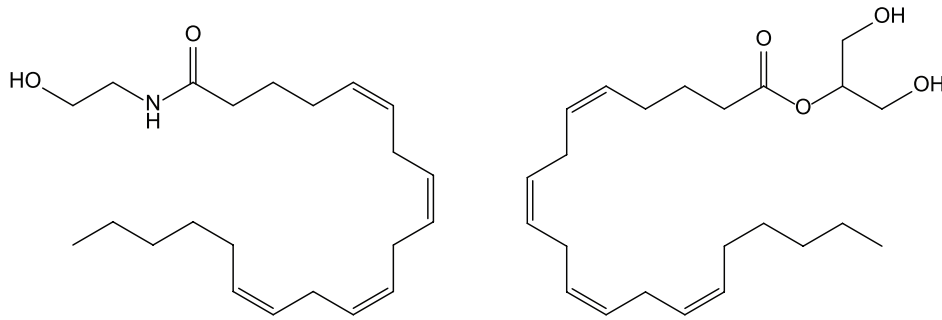
Figure 7. The endocannabinoids arachidonoylethanolamine (anandamide, left) and 2-arachidonoylglycerol (2-AG, right).

spectrum of activity at hydrolases compared with more selective FAAH inhibitors (such as PF-3845 by Pfizer and JNJ-42165279 by Janssen),[81] the underlying biochemical mechanisms involved in the toxic cerebral syndrome remain largely unknown.[82]

2-Arachidonoylglycerol is also an integral signaling component of lipid membranes but is produced, liberated, and degraded in a manner different from that of anandamide. In neuronal tissues, the synthesis of 2-AG begins with the precursor phosphatidylinositol 4,5-bisphosphate and proceeds via a diacylglycerol (DAG) intermediate that is cleaved to 2-AG by one of 2 diacylglycerol lipases, DAGLα or DAGLβ.[83,84] It appears likely that the DAG intermediate of 2-AG is synthesized on demand following

- Depolarization-induced activation of voltage-gated Ca^{2+} channels, wherein the increased intracellular Ca^{2+} concentration facilitates cleavage of phosphotidylcholine (PC) by phospholipase D to phosphatidic acid (PA), and subsequent removal of the phosphate group via PA phosphatase, to yield DAG;
- Activation of Gq/11-coupled receptors, such as metabotropic glutamate receptors and muscarinic acetylcholine receptors, coupled to phospholipase C (PLCβ)-mediated generation of DAG;
- Increase in DAG through activation of PLC_γ-coupled tyrosine kinase–linked growth factor receptors;
- Combinations of these biochemical pathways.[85,86]

Most of the DAG in the brain is enzymatically degraded by monoacylglycerol lipase (MAGL), and even modest systematic inhibition of MAGL can profoundly enhance 2-AG concentrations and effects. Acute inhibition of MAGL activity potentiates CB_1 receptor signaling[87] and produces agonist-like effects in laboratory animals.[88] However, both chronic pharmacological inhibition[89] and genetic deletion[90] of MAGL produce functional reductions in CB_1 receptor signaling (ie, β-arrestin 2–mediated desensitization) rather than activation.[86,91] The alpha/beta domain–containing hydrolase 6, alpha/beta domain–containing hydrolase 12, COX-2, and FAAH enzymes can also participate in the degradation of 2-AG.[92,93]

The Multifaceted Functioning of the Endocannabinoid System

The neuronal endocannabinoid system involves the concerted actions of endocannabinoids, cannabinoid receptors, and the enzymes responsible for the synthesis and degradation of endocannabinoids. In the CNS, most CB_1 receptors function to modulate neurotransmitter release and are present in particularly high concentrations in preterminal axon segments and axon terminals while being sparsely distributed in the active zone of the synapse[94] (Figure 8). The enzymes involved in endocannabinoid biosynthesis and degradation are also heterogeneously distributed throughout the brain in a manner consistent with their function.[95] In the hippocampus, for example, the DAGLα isoform is concentrated on dendritic spine heads of pyramidal cells and produces 2-AG that participates in retrograde synaptic signaling with presynaptic CB_1 receptors located on afferent terminals, which serves to adjust neurotransmitter release as a function of postsynaptic activity.[96] Monoacylglycerol lipase is found in laminar and punctate staining patterns on the afferent axon terminals instead of the postsynaptic structures at the ultrastructural level, demonstrating the post- and presynaptic segregation of the primary enzymes responsible for synthesis and elimination of 2-AG, respectively, in the human hippocampus. The anatomical arrangement is consistent with the endocannabinoid-mediated retrograde regulation of neuronal activity and synaptic plasticity in the cortex, hippocampus, amygdala, and dorsal striatum involved in executive function, perception, integration, learning, memory, extinction, recall, and habit.[86] Inhibition of synaptic transmission by CB_1 agonists has been demonstrated in diverse regions of the central and peripheral nervous systems, involving glutamatergic, GABAergic, noradrenergic, cholinergic, and other neurotransmitters.[97] For instance, the cannabinoid receptor also functions in the peripheral (although still neuronal) and central (spinal and supraspinal) levels in the pain pathway. Even at the subcortical level where CB_1 density is relatively sparse, the endocannabinoid system regulates the release of hypothalamic orexigenic and anorexigenic signals and modulates activity in mesolimbic dopaminergic incentive pathways and opioidergic hedonic circuits controlling motivation for and perceived value of rewards such as palatable food.[98] The CB_2 receptor is expressed to a much lower extent in
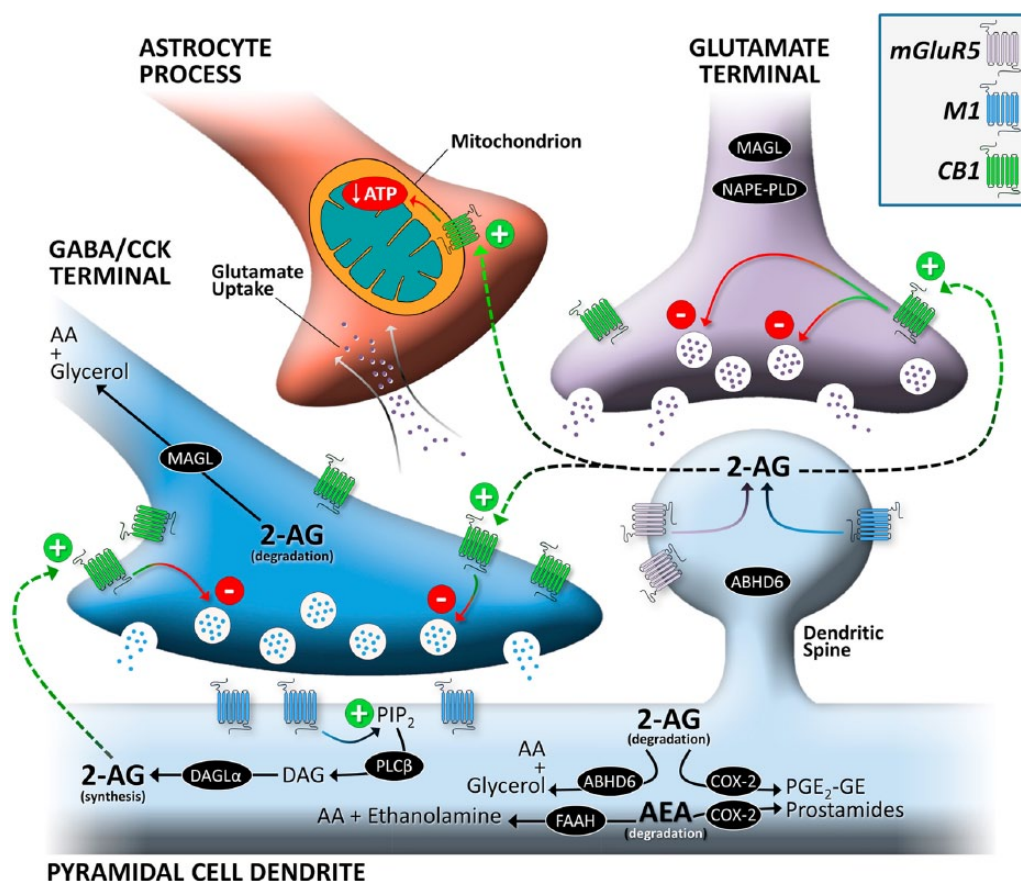
Figure 8. Neuronal endocannabinoid system. Reproduced with permission from Lu and Mackie.[100]

neurons in the mammalian CNS. In particular, CB_2 messenger RNA and receptor protein have been found in several brain stem regions, including the dorsal motor nucleus of the vagus, the nucleus ambiguus, and the spinal trigeminal nucleus. Although CB_2 signaling has putative roles in addiction and behavior, its impact in CNS functions and pathologies is less certain than that of the CB_1 system.[99]

Components of the endocannabinoid system are also distributed throughout the periphery and in several nonneuronal tissues, where they participate in a range of physiological and pathological processes. For instance, endocannabinoid signaling in the gastrointestinal tract, adipocytes, liver, kidney, skeletal muscle cells, and pancreas contributes to the control of intestinal motility, nutrient intake, energy storage, metabolism, and elimination processes involved in homeostasis.[101,102] In the male and female reproductive systems, endocannabinoid signaling functions along with hormones in the control of fertility.[103] Cannabinoid receptors are also found on several immune cell types and within organs (eg, spleen and thymus) with immune system function[14,104] that can produce and degrade endocannabinoids. Endocannabinoid tone and exogenous cannabinoid agents such as Δ^9-THC modulate cytokine (eg, tumor necrosis factor-α, interleukin-1β, interleukin-6, interleukin-12, and interleukin-10) and chemokine production, the expression of adhesion molecules, and the migration, proliferation, and apoptosis of cells.[105,106] Thus, the endocannabinoid system also

provides an integral component in the regulation of the cell cycle, immune system function, and inflammation. For example, the expression of CB_2 is increased in activated astrocytes, reactive microglia, oligodendrocytes, perivascular microglia, and neural progenitor cells, making the CB_2 receptor a particularly interesting biochemical target for the development of therapeutics for cancer, neuroinflammatory and neurodegenerative diseases, ischemic trauma, and stroke.[107]

Conclusions and Future Directions

Many advances in our understanding of the receptors, biochemicals, and enzyme systems composing the endocannabinoid system have been made in the past few decades. Because of the widespread distribution and diverse functioning of the system components, the effects observed during its modulation or dysregulation can be numerous and varied. The neuromodulatory role of the system and its actions in the control of energy balance (at the level of both food intake/satiety and peripheral storage and metabolism) are two of the most well-characterized effects observed in humans. Individuals increasing their endogenous cannabinoid tone with cannabis or cannabinoids experience altered sensations, perceptions, cognition, learning, memory, and behavior. The pharmacological effects of either herbal cannabis or Δ^9-THC vary in intensity with dose and route of administration and are modulated to some degree by the modest affinity and efficacy of the partial

agonist Δ^9-THC. The subjective effects are often perceived as pleasant or euphoric, but increased appetite and antiemetic effects are the only clinically approved therapeutic indications for synthetically derived Δ^9-THC (as dronabinol, trade name Marinol) in the United States, and it remains a scheduled substance because of its abuse liability. Consumption of illicit herbal products (eg, "Spice") containing synthetic cannabimimetic alkylindoles with high affinity and efficacy at CB_1 and CB_2 receptors can produce extreme alteration in the subjective effects observed in humans, leading to panic, confusion, anxiety, and other undesirable effects quite dissimilar from Δ^9-THC. Chronic repeated exposure to cannabinoid agonists can alter receptor signal transduction and trafficking pathways (eg, receptor internalization and downregulation/desensitization) in a process of adaptation that can lead to tolerance, dependence, and withdrawal upon abstinence. CB_1 receptor expression levels and availability in homeostatic and mesolimbic reward regions are inversely related to body mass index across individuals with and without food intake disorders.[98] Obese individuals decreasing their endogenous cannabinoid tone with the human-use formulation of the inverse agonist SR141716A (rimonabant) tended to lose weight and have improved biological markers of diabetes mellitus.[108–111] However, the drug was removed from the market because of treatment-associated depression and suicidal ideation that could be associated with its inverse agonist activity in the mesolimbic reward system.[98,109,110,112,113]

It should be emphasized that the integration and function of the various components of the endocannabinoid system are complex and that modulation influences diverse physiological processes that continue to be identified and further characterized. As a result, our understanding of drug use, addiction, obesity, and other pathological conditions for which endocannabinoid-based therapeutics might be targeted continues to increase dramatically. Moreover, because of the multifaceted functioning of the system, opportunities for advances in therapeutic agents that directly or indirectly affect CB_1 and CB_2 receptors (eg, medicinal cannabis and synthetic Δ^9-THC) are unlikely to provide clinical advantage in the absence of unwanted side effects. Nevertheless, it should also be recognized that the continued investigation of the endocannabinoid system provides a knowledge-based foundation for cannabis use/abuse and the development of more selective, effective, safe therapeutic agents. For example, compounds that selectively target CB_1 but do not cross the blood-brain barrier could eliminate CNS effects, such as abuse liability, yet retain peripheral effects on energy storage and utilization. Therefore, further investigation and development of endocannabinoid system modulators appear to be well -warranted and promising.

Author Contributions
BFT conceived and wrote the brief review.

Disclosures and Ethics
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