Chapter 65

Cannabinoid Agonists

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Abbreviations

2-AG 2-Arachidonoylglycerol
CNS Central nervous system
COX Cyclooxygenase
DAGL Diacylglycerol lipase
DSE Depolarization-induced suppression of excitation
DSI Depolarization-induced suppression of inhibition
FAAH Fatty acid amide hydrolase
HPA Hypothalamic–pituitary–adrenocortical
LOX Lipoxygenase
LTD Long-term depression
MAG Monoacylglycerol
MAGL Monoacylglycerol lipase
NADA *N*-Arachidonoyldopamine
NAE *N*-Acylethanolamine
NAPE *N*-Acylphosphatidylethanolamine
NAPE-PLD *N*-Acylphosphatidylethanolamine-hydrolyzing phospholipase D
NArPE *N*-Arachidonoylphosphatidylethanolamine
NAT *N*-Acyltransferase
PLC Phospholipase C
PNS Peripheral nervous system
TRPV1 Transient receptor potential cation channel subfamily V member 1
Δ^9-THC Δ^9-Tetrahydrocannabinol

INTRODUCTION

Cannabis has been cultivated by humans for the longest time. It is not known when or where the first plant arose, although it is thought to have happened in Asia. All throughout history it has been used by humankind for several purposes: in industry, using its fiber and obtaining oil from its seeds, but also in medical and religious contexts.

Cannabinol was the first component of cannabis to be isolated at the end of the nineteenth century (Wood, Spivey, & Easterfield, 1899), but its full chemical structure was not clarified until the mid-twentieth century (Adams, Baker, et al., 1940). That same year (1940), cannabidiol was also purified (Adams, Hunt, et al., 1940).

In the 1940s and 1950s the first cannabinoid agonists were synthesized, but research on these molecules was ceased because of the contradicting results obtained from various studies and the psychoactive effects they induced.

The real revolution in cannabis research came in the 1960s with the characterization of Δ^9-tetrahydrocannabinol (Δ^9-THC), the cannabinoid agonist responsible for the psychoactive effects of cannabis (Gaoni & Mechoulam, 1964). This finding led to further research with the objectives of understanding the medical properties of cannabis, synthesis of new cannabinoid agonists, and discovery of the endocannabinoid system.

Depending on their origin, cannabinoid agonists can be classified as:

- Endogenous substances found in the animal organism that are part of the endocannabinoid system (endocannabinoids),
- Molecules that can be found in the plant (phytocannabinoids),
- Molecules that are synthesized in the laboratory (synthetic cannabinoids).

ENDOCANNABINOIDS

The endocannabinoid system is formed by several elements: at least two specific receptors named cannabinoid receptors; the endogenous ligands of these receptors, also called endocannabinoids; and several enzymes and proteins that regulate ligand concentration.

An Introduction to the Endocannabinoid System

The first component of the endocannabinoid system to be discovered was the CB1 receptor. This receptor was first cloned from the brain of the rat (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990), sometime after from humans (Gerard, Mollereau, Vassart, & Parmentier, 1991), and 5 years later from the mouse (Chakrabarti, Onaivi, & Chadhuri, 1995). Overall they share 97–99% sequence identity (Howlett et al., 2002) and similar distributions throughout the species (Herkenham et al., 1990).

Three years after the CB1 receptor was cloned, a second cannabinoid receptor was identified from human promyelocytic cells and was named CB2 (Munro, Thomas, & Abushaar, 1993). It presents a slightly lower homology in the sequence of amino acids between humans, rats, and mice: 81% and 82%, respectively (Griffin, Tao, & Abood, 2000; Shire et al., 1996).

Both receptor types are members of the seven-transmembrane-domain G-protein-coupled family. They share 44% homology in their amino acid sequences and 68% when only the transmembrane region is considered (Munro et al., 1993). The main differences between these receptors are the way the signals are transduced to the interior of the cell and their distribution in the various tissues (Howlett et al., 2002).

When cannabinoid receptors were discovered it was thought that the CB1 receptor was exclusively located within the central nervous system (CNS) and CB2 in the periphery. Nowadays it is known that these differences in location are not so strict. CB1 is mainly expressed within the CNS; this receptor is the most abundant G-protein-coupled receptor in the brain, with especially high levels in the striatum, cerebellum, basal ganglia, cerebral cortex, and hippocampus (Herkenham et al., 1991). In the peripheral nervous system it is also found, but to a lesser extent. In peripheral non-neural tissues it has been described to be in adrenal gland, adipose tissue, heart, liver, lung, prostate, uterus, ovary, testis, bone marrow, thymus, and tonsils (Galiegue et al., 1995). The widespread distribution of the CB1 receptor is consistent with the multiplicity of effects of cannabinoid agonists, including hypomotility, increased food intake, disruption of short-term memory consolidation, antinociception, deficits of executive function, anxiety/anxiolysis, and psychotropic effects.

On the other hand, the CB2 receptor is mostly, though not exclusively, expressed in peripheral tissues, especially in immune tissues. This receptor is mainly expressed in spleen, tonsils, and thymus, tissues responsible for immune cell production and regulation. These immune cells include mast cells, B cells, T4 and T8 cells, microglial cells, macrophages, natural killer cells, and, to a lesser extent, monocytes and polymorphonuclear neutrophils (Howlett et al., 2004).

As stated before, the first studies indicated that CB2 receptors were not present in neurons of the CNS, although in past years they have been localized in several structures of the CNS: mRNA has been found in cortex, hippocampus, cerebellum, and brainstem (Van Sickle et al., 2005). A more detailed review of the distribution of CB1 and CB2 receptors in the CNS has been performed by Svizenska and coworkers (Svizenska, Dubovy, & Sulcova, 2008).

After the discovery of the CB1 receptor, Mechoulam and coworkers followed the reasoning that had led to the discovery of endogenous opioids: evolution could not have maintained in the organism a receptor for only a plant to stimulate; this led to the identification of the first endocannabinoid, *N*-arachidonoylethanolamine, which was named anandamide after the Sanskrit word *ananda,* which means bliss, joy, delight (Devane et al., 1992).

A few years later a second endocannabinoid was identified, 2-arachidonoylglycerol (2-AG), first in the intestine of the dog (Mechoulam et al., 1995) and then in the brain (Sugiura et al., 1995).

Since then other compounds have been isolated, such as 2-arachidonylglyceryl ether (noladin ether), *O*-arachidonoylethanolamine (virodhamine), and *N*-arachidonoyldopamine (NADA). However, the endogenous functions in physiological processes for all these compounds have not yet been established in detail and as of this writing, anandamide and 2-AG are the most studied endocannabinoids of the two major classes of endocannabinoids, *N*-acylethanolamines (NAEs) and monoacylglycerols, respectively. These compounds are frequently referred to as the "major" endocannabinoids (De Petrocellis & Di Marzo, 2009).

Endocannabinoid Agonists in the CNS

The levels of endocannabinoids vary across the structures of the CNS, 2-AG being the most abundant endocannabinoid, in both naïve rats and postmortem human brain (Buczynski & Parsons, 2010; Palkovits et al., 2008).

Anandamide is a partial agonist for CB1 and CB2 receptors, though with slightly higher affinity for CB1. In contrast, 2-AG behaves as a full agonist for CB1 and CB2 receptors, with higher receptor potency at CB1 and CB2 than anandamide. Both show less affinity for CB2 than CB1 receptor (Pertwee et al., 2010).

The available literature gives CB1 a predominant role as the major target for endogenous and exogenous cannabinoids at axon terminals within the CNS, although emerging data are demonstrating a possible role of CB2 receptors in synaptic transmission (den Boon et al., 2012). Future experiments are needed to determine the participation of CB2 in neuronal activity.

One of the peculiar characteristics of endocannabinoids is that, when modulating synaptic transmission, they act in a retrograde way (although not exclusively). So, endocannabinoids are synthesized from the postsynaptic neuron and bind to the presynaptic cannabinoid receptor. The result of this binding is a presynaptic inhibition of excitatory or inhibitory neurotransmitter release in the same or a neighboring synapse, which can be short or long term.

Another peculiar characteristic is that endocannabinoids cannot be stored in vesicles as other neurotransmitters. Because of their lipophilic structure, they are synthesized on demand once the postsynaptic neuron is stimulated. Several different stimuli, such as an increase in intracellular calcium, activation of group I metabotropic glutamate receptors, and/or $G_{q/11}$-protein-coupled receptor stimulation, lead to the cleavage of membrane phospholipids and synthesis of endocannabinoids (Pagotto, Marsicano, Cota, Lutz, & Pasquali, 2006).

Endocannabinoid Metabolism

Although both endocannabinoids are synthesized using phospholipids as precursors the synthesis is achieved through distinct mechanisms.

Membrane glycerophospholipids are the precursor molecules of anandamide and other NAEs. Their biosynthesis can be subdivided into two different phases: the first resulting in the respective *N*-acylphosphatidylethanolamine (NAPE) and the second giving the final NAE.

In the case of anandamide the first reaction consists of the synthesis of *N*-arachidonoylphosphatidylethanolamine (NArPE). This is obtained by two Ca^{2+}-dependent reactions, a first one in which diarachidonoylphosphatidylcholine is formed and a second to form NArPE. The second phase in the synthesis of anandamide consists of the hydrolysis of NArPE, resulting in anandamide and phosphatidic acid. The enzyme responsible for the first reaction is an *N*-acyltransferase (NAT) and the second reaction is catalyzed by the *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD). While the pathways of NArPE synthesis seem to be selective for anandamide, although the molecular characterization of the NAT responsible for these reactions has yet not been achieved, NAPE-PLD does not distinguish between NAPEs.

Two other pathways for anandamide synthesis have been proposed; both start off with NArPE. In one of these pathways an

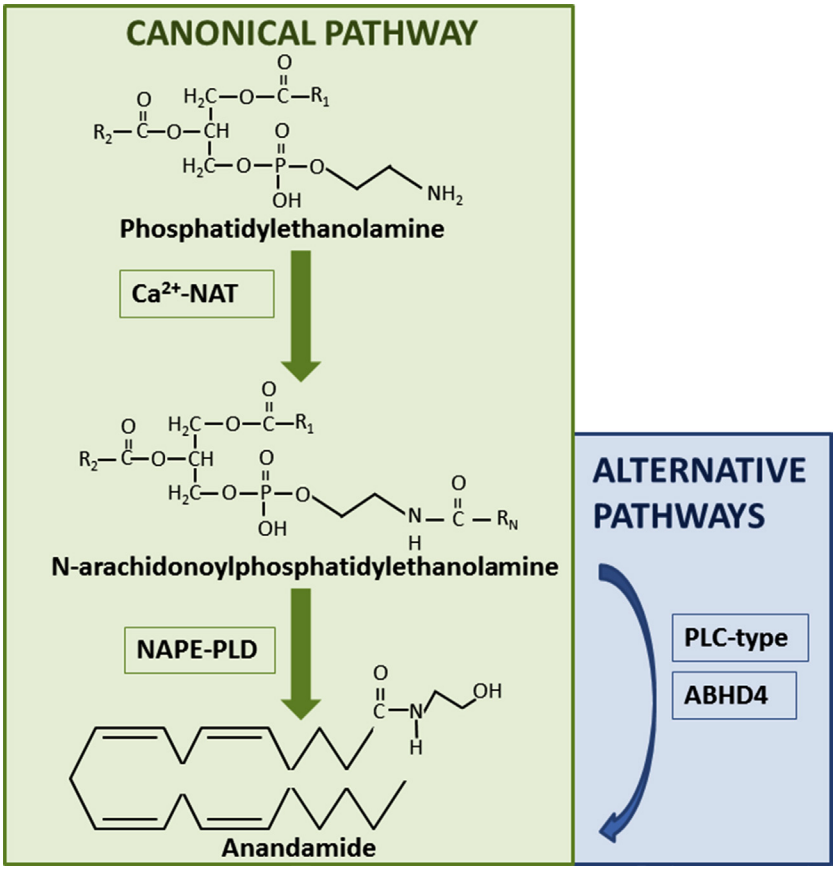

FIGURE 1 **Canonical and alternative pathways for anandamide synthesis.** The first reaction consists of the synthesis of NArPE by the enzyme NAT. Then NARPE is hydrolyzed by NAPE-PLD, releasing anandamide and phosphatidic acid. Two other alternative pathways have been proposed in which an enzyme with PLC activity and the enzyme ABHD4 are implicated.

enzyme with phospholipase C (PLC) activity converts NArPE into phosphoanandamide, and a phosphatase activity cleaves phosphoanandamide into anandamide and free phosphate. In the second pathway proposed, first glycerophosphoanandamide is catalyzed by the enzyme α/β hydrolase domain-containing protein 4 (ABHD4), which may be transformed into anandamide by cleavage of its phosphodiester bond, catalyzed by the phosphodiesterase GDE1, with release of glycerol phosphate (Piomelli, 2014; Rahman, Tsuboi, Uyama, & Ueda, 2014; Reisenberg, Singh, Williams, & Doherty, 2012) (Figure 1).

In contrast to the synthesis of anandamide, the routes by which 2-AG are formed seem to be better understood. The most important route by which 2-AG is synthesized is through the hydrolysis of 2-arachidonoylphosphatidylinositol by the PLC enzyme to form 2-arachidonoyldiacylglycerol, which is then hydrolyzed by diacylglycerol lipase (DAGL) to 2-AG (Ueda, Tsuboi, & Uyama, 2013).

Among PLC enzymes, PLC-β can be activated by several $G_{q/11}$-protein-coupled receptors. This suggests that activation of these receptors by neurotransmitters in postsynaptic neurons activates PLC-β, which will consequently synthesize 2-AG. DAGL is a membrane-associated enzyme, which can be stimulated by Ca^{2+}; two subtypes have been implicated in 2-AG formation: DAGLα and DAGLβ. Their contribution to 2-AG is tissue dependent; DAGLα appears to be more important in brain tissues and spinal cord (Piomelli, 2014; Rahman et al., 2014; Reisenberg et al., 2012; Ueda et al., 2013).

As with anandamide, an alternative pathway for 2-AG synthesis has been proposed: a first step mediated by the phospholipase A1, which gives 2-arachidonoyllysophosphatidylinositol, and a final reaction in which the enzyme lyso-PLC hydrolyzes 2-arachidonoyllysophosphatidylinositol into 2-AG (Piomelli, 2014; Rahman et al., 2014; Reisenberg et al., 2012; Ueda et al., 2013) (Figure 2).

Cannabinoid degradation occurs within the cell, so once endocannabinoids are shed to the synaptic cleft they must be transported to the intracellular space. Three different mechanisms have been proposed for the transportation of anandamide through the membrane: passive diffusion, facilitated transport, and internalization through caveolae. Once anandamide is internalized, evidence demonstrates the existence of intracellular binding proteins, which might allow its transport through the cytosol (Maccarrone, Dainese, & Oddi, 2010).

Anandamide is metabolized by the fatty acid amide hydrolase (FAAH) into arachidonic acid and ethanolamine (Cravatt et al., 1996). This enzyme is a membrane-bound serine hydrolase, although unlike other serine hydrolases it possesses an atypical catalytic triad consisting in Ser–Ser–Lys instead of the Ser–His–Asp.

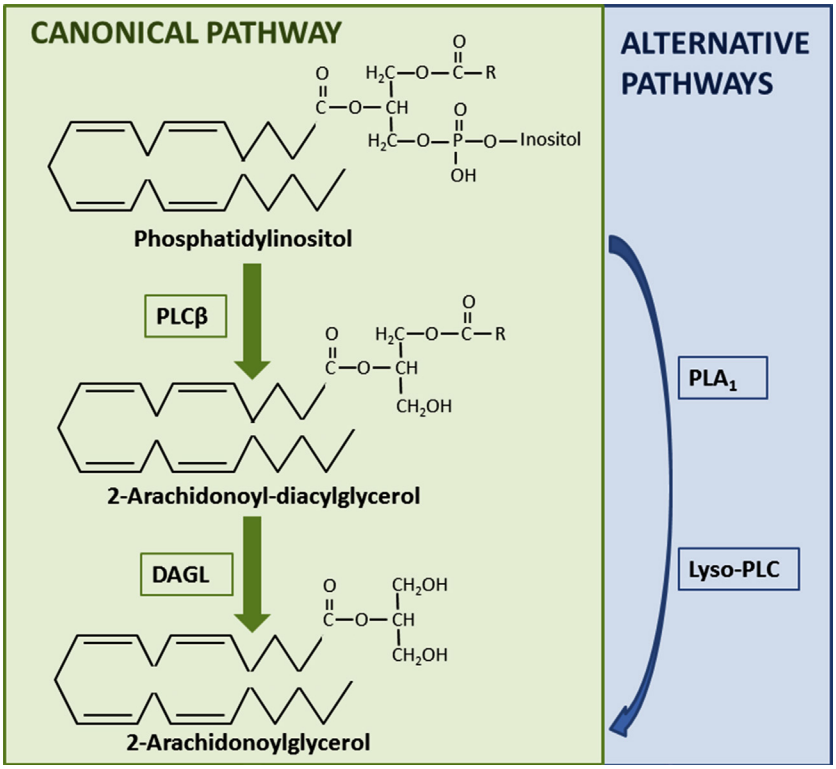

FIGURE 2 **Canonical and alternative pathways for 2-AG synthesis.** The most important route by which 2-AG is synthesized is through the hydrolysis of 2-arachidonoylphosphatidylinositol by the PLC enzyme to form 2-arachidonoyldiacylglycerol, which is then hydrolyzed by DAGL to 2-AG. An alternative pathway for 2-AG synthesis has been proposed: a first step mediated by the phospholipase A1 (PLA_1), which gives 2-arachidonoyllysophosphatidylinositol, and a final reaction in which the enzyme lyso-PLC hydrolyzes 2-arachidonoyllysophosphatidylinositol into 2-AG.

FAAH is formed by a large domain that anchors the enzyme to the membrane; a channel, which permits the entry of the substrate; a hydrophobic cavity, which interacts with the side chain of the substrate; and a cytosolic port, which interacts with the polar head of the substrate and is connected with the cytosol (Feledziak, Lambert, Marchand-Brynaert, & Muccioli, 2012).

A second isoform (FAAH-2) has been identified in mammals, but not in rodents. Both enzymes share 20% amino acid sequence identity, though their orientation in the membrane is different. Also their distribution patterns vary: FAAH-1 is preferentially expressed in brain, testis, and small intestine, while FAAH-2 is predominant in cardiac tissue (Yates & Barker, 2009).

The main enzyme responsible for the degradation of 2-AG is monoacylglycerol lipase (MAGL). MAGL breaks down 2-AG into glycerol and arachidonic acid, which is then incorporated into the membrane or transformed into the eicosanoid family of lipid mediators. It belongs to the serine hydrolase superfamily and is localized in the cytosol. 2-AG can also be broken down by other enzymes such as cyclooxygenase-2, lipoxygenase, or FAAH. It has a heterogeneous distribution in the rat brain with the highest expression in the regions where the CB1 receptor is most abundant, such as the hippocampus, cortex, and cerebellum (Ueda et al., 2013).

Endocannabinoids and Synaptic Modulation

Once endocannabinoids are synthesized, 2-AG and anandamide are shed to the synaptic cleft by a mechanism that is, as of this writing, unknown, and bind to the presynaptic CB1 receptor (Figure 3). This binding results in a short-term inhibition of γ-aminobutyric acid (GABA) or glutamate release, which persists for tens of seconds. This phenomenon was termed, depending on the neurotransmitter inhibited, depolarization-induced suppression of inhibition (DSI), when GABA release is inhibited, or depolarization-induced suppression of excitation (DSE), when glutamate release is inhibited (Castillo, Younts, Chavez, & Hashimotodani, 2012; Melis, Greco, & Tonini, 2014).

In addition to the short-term inhibition of neurotransmitter release, it has also been proven that endocannabinoids can mediate presynaptic forms of long-term depression (LTD) at both excitatory and inhibitory synapses. This can be induced by both anandamide and 2-AG. It is thought that the postsynaptic mechanisms leading to LTD are similar to DSE/DSI, although presynaptic proteins such as Rab3B/R1M1α or a reduction in P/Q-type voltage-gated channels may be involved. Also, in DSI/DSE there is an inhibition of presynaptic calcium influx through Ca^{2+} voltage-gated channels, probably via the βγ subunits, while in LTD, inhibition of adenylyl cyclase and downregulation of the cAMP/protein kinase A pathway via the $\alpha_{i/o}$ limb are required (Castillo et al., 2012; Melis et al., 2014).

With the introduction of different pharmacological tools (such as FAAH or MAGL inhibitors) and genetically altered mice, the specific roles of these two major endocannabinoids in the CNS are being studied. Both endocannabinoids have been shown to be involved in neuroprotection, learning and memory, nociception,

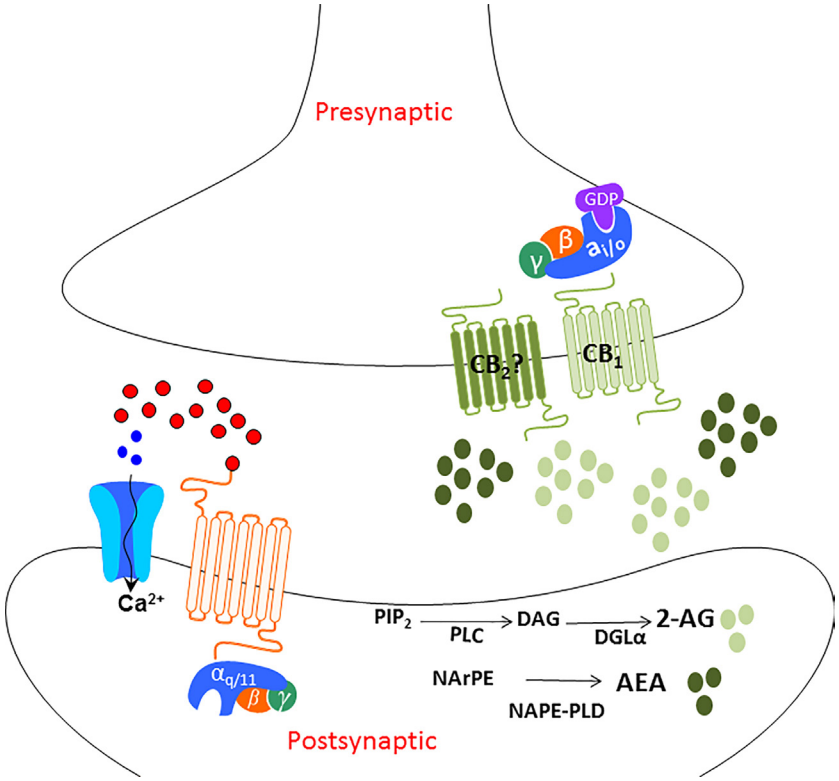

FIGURE 3 Schematic of endocannabinoid synthesis and retrograde signaling. Postsynaptic increase in Ca^{2+} influx or activation of $G_{q/11}$ induces endocannabinoid synthesis. Endocannabinoids are then shed to the synaptic cleft and bind to the CB1 receptor (the role of the CB2 receptor is yet to be determined), which consequently inhibit GABA and glutamate release.

anxiety, reward, and drug addiction, although the participation of either endocannabinoid in each function is different. 2-AG is the main endocannabinoid responsible for most forms of DSI/DSE and some forms of LTD, while anandamide mediates certain forms of synaptic homeostasis and plasticity. Anandamide and 2-AG take part in highly specialized compartments of physiological and pathophysiological functioning; even when they cooperate anandamide and 2-AG take part in different steps of the same function, although how these two endocannabinoids interact is yet fairly unknown. For a review, see Luchicchi and Pistis (2012).

An example of how anandamide and 2-AG interact is observed in the modulation of acute stress. Studies have demonstrated that there is a tonic endocannabinoid tone in the prefrontal cortex and amygdala, induced mainly by the binding of anandamide to CB1, inhibiting the hypothalamic–pituitary–adrenocortical (HPA) system. Under stimuli of acute stress there is a rapid reduction in anandamide levels due to an increased activity of FAAH. When the stimulus is ceased there is a rapid increase in 2-AG, which has been proposed to have an inhibitory effect over the HPA system and in consequence the recovery from the stressful stimuli (Gunduz-Cinar, Hill, McEwen, & Holmes, 2013).

Under conditions of chronic stress anandamide is persistently reduced. It is not clear if this reduction is due to an increase in FAAH activity or disruption in the synthesis of anandamide. This reduction in anandamide induces an impaired cannabinoid signaling hyperactivation of the HPA axis and consequently an increased vulnerability to stress-related illnesses (Gunduz-Cinar et al., 2013).

All the physiological effects of endocannabinoids cannot be fully understood through their binding to the CB1 and CB2 receptors, however. Anandamide can also activate different receptors, such as the peroxisome proliferator-activated receptors or the transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor.

The TRPV1 receptor belongs to the transient receptor potential family, which is composed of six subfamilies. Receptors belonging to three of these subfamilies have been suggested to interact with cannabinoid agonists: TRPV1, TRPV2, and TRPV4 (belonging to the vanilloid subfamily), TRPM8 (belongs to the melastatin subfamily), and TRPA1 (belongs to the ankyrin subfamily).

TRPV1 is a nonselective cation channel that is activated by noxious heat (>43 °C), low pH (<6.9), and capsaicin (pungent agent of chili peppers) and consequently induces nociception, although it is also expressed in the brain, where it has been shown to participate in antinociception (Kim et al., 2012), locomotor control (Lee, Di Marzo, & Brotchie, 2006), and regulation of affective behaviors (Moreira, Aguiar, Terzian, Guimaraes, & Wotjak, 2012).

Anandamide, but not 2-AG, acts as a full agonist; its affinity is similar or slightly lower than that of capsaicin, and it seems to interact with TRPV1 at the same intracellular binding site as capsaicin (Pertwee et al., 2010).

TRPV1 colocalizes with CB1 and CB2 in sensory neurons and with CB1 in several brain areas and CB2 in osteoclasts. Anandamide can bind to both presynaptic and postsynaptic TRPV1 receptors. When binding to the postsynaptic TRPV1 receptor it will induce hyperpolarization of neurons, whereas when binding

to the presynaptic TRPV1 it will facilitate glutamatergic signaling. But how these two receptors interplay to modulate synaptic transmission is, as of this writing, not completely clarified (Di Marzo & De Petrocellis, 2012).

Other endogenous cannabinoids that have been shown to bind to the TRPV1 receptor are NADA with a higher potency than anandamide or *N*-oleoyldopamine (Pertwee et al., 2010).

Less is known about the action that endocannabinoids exert over other TRP channels. Along this line anandamide and NADA have been shown to antagonize the effects of the TRPM8 agonists menthol and icilin and weakly activate the TRPA1 channel (De Petrocellis & Di Marzo, 2009; De Petrocellis et al., 2012), although future experiments are needed to determine the physiologic functions they exert.

The orphan G-protein-coupled receptor GPR55 has also been proposed to mediate some of the effects of endocannabinoids and Δ^9-THC. This protein is highly expressed in the brain and peripheral tissues, although it presents little homology to either cannabinoid receptor already cloned. The results are contradicting and future experiments are needed (De Petrocellis & Di Marzo, 2009).

PHYTOCANNABINOIDS: Δ^9-TETRAHYDROCANNABINOL

Nowadays it is known that the *Cannabis sativa* plant has around 400 compounds, and more than 60 different cannabinoids have been identified. The main cannabinoids present in the plant are Δ^9-THC, Δ^8-THC, cannabidiol, and cannabinol. Although all of them, except for cannabidiol, have psychoactive effects, Δ^9-THC is the molecule responsible for most of the psychoactive effects of cannabis. All are extremely hydrophobic molecules.

The pharmacokinetics of Δ^9-THC depends on the method of its administration. When smoked, Δ^9-THC is rapidly absorbed into the bloodstream, and maximal concentration is reached within a few minutes. In contrast, when orally administered, Δ^9-THC has a lower bioavailability, as it is sensitive to gastric acid and hepatic metabolism, reaching maximum plasma concentrations within 1–2 h. In plasma 60% of the drug is associated with the lipoprotein; the remainder of the drug appears to be bound by albumin and blood cells. Δ^9-THC is initially distributed to highly perfused organs such as the heart, kidneys, lung, placenta, or liver and then into adipose tissue, where it can remain and take several weeks to be totally eliminated, though its retention in this tissue reduces its penetration into the CNS (its presence in the CNS is usually around 1% of the maximum plasma concentration). Most metabolites are excreted through feces (68%) and urine (12%), although also through hair, saliva, and sweat.

Most Δ^9-THC metabolism occurs in the liver, where it is hydroxylated to 11-hydroxy-Δ^9-THC, although it can also be metabolized in the lung or intestine. Levels of this metabolite can be detected minutes after smoking Δ^9-THC and reach maximum plasma concentrations 30–60 min after smoking. As it is also a psychoactive metabolite, it could potentiate the effects of Δ^9-THC in the CNS (Gonzalez, Sagreso, et al., 2002; Huestis, Mazzoni, & Rabin, 2011).

Like anandamide, Δ^9-THC is a partial agonist for CB1 and CB2 receptors; it resembles anandamide in its CB1 affinity, although with less efficacy than anandamide and with less relative intrinsic activity at CB2 than at CB1 receptors. Cannabidiol displays a very low affinity for CB1 and CB2 receptors; despite this low affinity cannabidiol can interact with these receptors at low concentrations and block several of the in vivo effects of Δ^9-THC (Pertwee, 2008; Pertwee et al., 2010). This has important clinical implications, for example, the neurocognitive and psychological effects after sporadic or long-term use of cannabis are likely to vary depending on the proportion of Δ^9-THC and cannabidiol present in the drug. Currently the strains that are high in Δ^9-THC and low in cannabidiol dominate the market (Schoeler & Bhattacharyya, 2013).

When used as a drug of abuse, Δ^9-THC can alter short-term memory, sense of time, attention, problem solving, verbal fluency, reaction time, and psychomotor control and induce positive feelings as mild euphoria and relaxation or, on the contrary, anxiety, paranoia, and panic reaction. These opposing effects are dose and context dependent: in general, low doses of Δ^9-THC exert anxiolytic and mood-enhancing effects, whereas high doses are anxiogenic and dysphoric. However, these dose-dependent effects also vary with other factors, including environment and previous experience with the drug (Clapper, Mangieri, & Piomelli, 2009). But the molecular mechanisms by which Δ^9-THC induces these antagonistic effects are yet not completely known. Like endogenously released cannabinoid agonists, Δ^9-THC can act through neuronal presynaptic CB1 receptors to inhibit ongoing neurotransmitter release onto other neurotransmitter-releasing neurons, consequently having a mixed stimulatory–inhibitory effect on neurotransmitter release. On the other hand, in some assays, it has been found that Δ^9-THC can also behave as a CB1 and CB2 receptor antagonist of exogenous and endogenous cannabinoids at these receptors. The way Δ^9-THC can act either as an agonist or as an antagonist is not fully clear, although the hypothesis exists that because Δ^9-THC has a relatively low cannabinoid receptor efficacy, its capacity to activate these receptors will be influenced by the density and coupling efficiencies of these receptors (Pertwee, 2008).

The effects of Δ^9-THC vary depending on the individual's history of Δ^9-THC abuse, that is, chronic use of Δ^9-THC has shown to induce tolerance (in which either the potency or the efficacy of a drug changes such that the physiological and/or psychological consequences of the same drug dose are significantly diminished with repeated use), both in humans and in laboratory animals. Although tolerance to the various effects of cannabinoids does not occur at the same time, in animals it has been shown that tolerance to motor impairment or hypothermia appears within the first 3–7 days of treatment, while neuroendocrine or memory impairment needs a much longer time of treatment (Gonzalez, Cebeira, & Fernandez-Ruiz, 2005). As in animals, tolerance to the various effects of cannabinoids also appears in humans at different time points (D'Souza et al., 2008).

The mechanism by which tolerance occurs has not been completely elucidated. It has been shown that downregulation (loss of receptors) and desensitization (attenuated receptor-mediated G-protein and effector activity) of the CB1 receptor occur and contribute to tolerance after chronic Δ^9-THC administration in animals (Lazenka, Selley, & Sim-Selley, 2013). Findings in humans and in animals have shown that downregulation after chronic exposure to Δ^9-THC does not occur in equal ways along the various structures of the CNS; CB1 receptors in the hippocampus suffer adaptations faster, while changes in striatum are slower. This could explain, partly, why tolerance to the different effects

induced by cannabis consumption appears at different time points (Hirvonen et al., 2012).

Previously it was thought that cannabis did not induce dependence, but it has been shown that approximately 10% of first-time users and 50% of cannabis users develop dependence. But the molecular basis responsible for the interindividual differences in becoming cannabis dependent is not known. Several factors, such as genetic variability, have been proposed. Interestingly, different genetic variants of the CB1 receptor and FAAH genes have been associated with an increase in susceptibility to drug addiction (Clapper et al., 2009; Oliere, Joliette-Riopel, Potvin, & Jutras-Aswad, 2013).

Ninety percent of cannabis dependents that go under treatment have a difficult time dealing with abstinence as they experience a withdrawal syndrome that is characterized by craving, irritability, anxiety, depressed mood, decreased appetite, and sleep difficulties and display similar scope and severity to the withdrawal associated with tobacco use. Interestingly, the affective and cognitive effects seem to have greater responsibility in cannabis relapse than craving. As of this writing, no specific therapeutic approaches have been developed for cannabis dependence and most interventions are based on those used for alcohol dependence. Studies in animals are investigating the use of anandamide transport and FAAH inhibitors for the treatment of cannabis dependence; the majority of evidence obtained so far suggests that indirect activation of CB1 receptors by increasing levels of synaptically available anandamide does not mimic the reinforcing effects typical of direct-acting cannabinoid agonists (Clapper et al., 2009; Maldonado, Berrendero, Ozaita, & Robledo, 2011).

SYNTHETIC CANNABINOIDS

Synthetic cannabinoids or cannabimimetic compounds were initially synthesized with the objective to target selectively CB1 or CB2 receptors for research purposes, although in the past few years there has been an increase in the intake of these drugs with an illicit purpose. Synthetic cannabinoids can be chemically classified into naphthoylindoles, benzoylindoles, phenylacetylindoles, adamantylindoles, cyclophenols, and a miscellaneous group (ElSohly, Gul, Wanas, & Radwan, 2014).

In the United States, synthetic cannabinoids first emerged on the drug market in 2008 and were marketed as "legal alternatives to marijuana," since the use of synthetic cannabinoids produces effects similar to those of cannabis. Since then 74 synthetic cannabinoids have been reported (United Nations Office on Drugs and Crime). This seems to be due to the manufacturer's intention to be one step ahead of legislation; as various cannabinoids are banned they are replaced by other compounds similar in structure and pharmacologically active. Unlike Δ^9-THC, the synthetic cannabinoids used are high-potency full agonists at the brain CB1 receptor (Spaderna, Addy, & D'Souza, 2013).

Hundreds of synthetic cannabinoids are categorized into the following structural groups: adamantoylindoles (e.g., AB-001), aminoalkylindoles (e.g., WIN 55,212-2), benzoylindoles (e.g., AM694), cyclohexylphenols (e.g., CP 55,940), dibenzopyrans (e.g., HU-210), naphthoylindoles (e.g., JWH-018), naphthylmethylindoles (e.g., JWH-175), naphthylmethylindenes(e.g., JWH-176), naphthoylpyrroles (e.g., JWH-307), phenylacetylindoles (e.g., JWH-250), tetramethylcyclopropyl ketone indoles (e.g., UR-144), quinolinyl ester indoles (e.g., PB-22), and indazole carboxamide compounds (Figure 1; Castaneto et al., 2014).

These synthetic cannabinoids have been found to be mixed with FAAH and MAGL enzyme inhibitors, cathinones, or tryptamines (Nakajima et al., 2013; Uchiyama, Kawamura, Kikura-Hanajiri, & Goda, 2013). For a review of emerging compounds used as drugs of abuse, see Lewin, Seltzman, Carroll, Mascarella, and Reddy (2014).

CANNABINOID AGONISTS IN THE CLINIC

As of this writing, three different cannabinoid drugs have been approved in different countries for the treatment of certain types of pain, anorexia, and nausea and vomiting.

The first was nabilone, a CB1/CB2 receptor agonist, which is a synthetic analogue of Δ^9-THC, indicated for the relief of nausea and vomiting produced by chemotherapy. For this purpose dronabinol was also approved some years later, although because 5-HT3 antagonists have higher effectiveness and better tolerability they are generally used instead. Dronabinol was introduced for the treatment of AIDS-related anorexia (Howard, Twycross, Shuster, Mihalyo, & Wilcock, 2013). Sativex® was approved in multiple countries for the treatment of neuropathic pain in patients with multiple sclerosis and patients with advanced cancer. Sativex® is composed of approximately equal amounts of dronabinol and cannabidiol.

A CB1 receptor antagonist, rimonabant, was introduced in the clinic for the treatment of obesity, although anxiety and depression symptoms were observed in a significant proportion of individuals, and a higher suicide risk, and it was removed from the market (Christensen, Kristensen, Bartels, Bliddal, & Astrup, 2007).

At present various strategies are being used to exploit the possible therapeutic effects of cannabinoid agonists, reducing the side effects mainly due to the activation of CNS CB1 receptors. These can be summarized as follows (Pertwee, 2009):

- Targeting cannabinoid receptors located outside the blood–brain barrier;
- Targeting cannabinoid receptors expressed by a particular tissue, which can be obtained by the local and topical administration of a cannabinoid agonist without reaching the CNS;
- Targeting upregulated cannabinoid receptors—some disorders trigger a "protective" upregulation of a subpopulation of CB1 and CB2 receptors that can mediate symptom relief or oppose disease progression;
- Multitargeting—this strategy relies on the capacity of cannabinoid agonists to act additively or synergistically with other systems (i.e., cannabinoid agonists are being studied in combined administration with opioids for the treatment of pain).

APPLICATION TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

The role of cannabinoid receptors in cocaine addiction has been studied, but few studies have aimed to investigate the implication of cannabinoid agonists in cocaine addiction.

Endocannabinoids play a central role in several cognitive and physiological processes associated with addiction, such as reward, stress responsiveness, and drug-related synaptic plasticity.

Acute and chronic administration of drugs has been shown to alter the endocannabinoid levels, i.e., acute administration of cocaine increases levels of 2-AG but not anandamide in the limbic forebrain (Patel, Rademacher, & Hillard, 2003). On the other hand, chronic cocaine exposure decreases 2-AG content of the limbic forebrain but not in other areas such as the cerebral cortex striatum or midbrain nor does it alter anandamide levels.

Endocannabinoid levels are not modified depending only on the time of drug abuse but also depending on the drug being abused. Chronic alcohol exposure has been shown to increase or decrease, in midbrain and limbic forebrain, the contents of anandamide and 2-AG, and nicotine induces changes in endocannabinoid contents in other structures such as the limbic forebrain, brainstem, hippocampus, and cerebral cortex (Gonzalez, Cascio, et al., 2002).

As of this writing, various preclinical studies aim to investigate cannabinoid receptor antagonists and FAAH inhibitors for the treatment of different addictions. The results obtained from these studies are controversial. In genetically modified mice without FAAH enzyme (FAAH knockout), or upon administration in mice of a FAAH inhibitor, nicotine withdrawal is increased. Interestingly, different results have been observed in rats in which inhibitors of FAAH inhibited nicotine reward, decreased reinstatement of nicotine-seeking behavior, and reversed nicotine withdrawal anxiety (Maldonado, Robledo, & Berrendero, 2013; Muldoon, Lichtman, Parsons, & Damaj, 2013).

Similar to what happens with nicotine, the results obtained in studies using cocaine and opioids vary among species. A pharmacological increase in anandamide levels reduced morphine withdrawal and reinstatement of cocaine-seeking behavior in rats and mice and did not reinstate extinguished drug-seeking behavior in monkeys that had previously self-administered cocaine (Justinova et al., 2008; Maldonado et al., 2013).

It seems without doubt that endocannabinoids are implicated in some aspects of drug addiction, such as in reward. Modulating endocannabinoid agonist levels could be a useful pharmacological option in the treatment of various addictions although future experiments are necessary.

DEFINITION OF TERMS

Cannabis *Cannabis* is an annual, dioecious, flowering herb that includes three different species, *Cannabis sativa, Cannabis indica,* and *Cannabis ruderalis*.

Agonist This is a chemical that binds to a receptor and activates the receptor to produce a biological response.

Endocannabinoids These are endogenous substances found in animal organisms that are part of the endocannabinoid system and bind to cannabinoid receptors.

Phytocannabinoids These are molecules that can be found in the cannabis plant or other plants and bind to cannabinoid receptors.

Synthetic cannabinoids These are molecules that are synthesized in the laboratory to bind selectively or not to one or more cannabinoid receptors.

Δ^9-Tetrahydrocannabinol This is the phytocannabinoid responsible for the psychoactive effects of cannabis.

Anandamide (*N*-arachidonoylethanolamine) This was the first endocannabinoid to be identified. It is a partial agonist for CB1 and CB2 receptors, with a slightly higher affinity for CB1.

2-Arachidonoylglycerol This was the second endocannabinoid to be identified. It is a full agonist for CB1 and CB2 receptors with a slightly higher affinity for CB1.

Fatty acid amide hydrolase This is an enzyme belonging to the serine hydrolase family and is responsible for metabolizing anandamide.

Tolerance This refers to when the potency or efficacy of a drug changes such that the physiological and/or psychological consequences of the same drug dose are significantly diminished with repeated use.

KEY FACTS

Key Facts of Cannabis

The first written references to the use of marijuana for medical purposes are found in the Chinese pharmacopoeia of Shen Nung (2600 BC) and Shen Nung (2700 BC).

The first scientific studies on the therapeutic effect of cannabis were published by Sir William B. O'Shaughnessy in 1839. The article described the analgesic, antispasmodic, and muscle-relaxing properties of cannabis. O'Shaughnessy's article roused interest in cannabis research, which led on to active cannabis research by other researchers.

Key Facts of Agonists

Agonists can behave as full agonists or partial agonists. When an agonist binds to the receptor and elicits a maximal response it is a full agonist, while partial agonists are not able to induce a maximal response.

Key Facts of Receptors

Receptors are protein molecules localized in the cell that are directly and specifically in charge of the chemical inter- and intracellular signaling.

There are various types of receptors; two of these types are ligand-gated ion channels and G-protein-coupled receptors. Ligand-gated ion channels are those in which the interaction of the agonist with the receptor causes the opening and closing of an ion channel in another part of the same molecule. G-protein receptors have seven transmembrane domains. These receptors are connected to the effector systems through GTP-binding proteins.

Key Facts of Endocannabinoid System

The endocannabinoid system is formed by at least two specific receptors named cannabinoid receptors; the endogenous ligands of these receptors, also called endocannabinoids, and several enzymes and proteins that regulate ligand concentration.

The endocannabinoid system modulates neurotransmission; endocannabinoids act primarily (though not exclusively) upon presynaptic receptors, which in turn inhibit the release of GABA or glutamate.

SUMMARY POINTS

- Cannabinoid agonists can be classified depending on their origin as endocannabinoids, phytocannabinoids, and synthetic cannabinoids.
- The most studied endocannabinoids are anandamide and 2-AG.
- Anandamide and 2-AG bind to presynaptic cannabinoid receptors to regulate synaptic transmission by inhibiting neurotransmitter release.
- Anandamide and 2-AG are synthesized from membrane phospholipids on demand, through different mechanisms that are not well established as yet. FAAH is the enzyme responsible for the metabolism of anandamide, and MAGL is the main enzyme responsible for 2-AG degradation.
- Anandamide and 2-AG can bind to other noncannabinoid receptors.
- Δ^9-THC and cannabidiol are phytocannabinoids. Δ^9-THC is the main molecule responsible for the psychoactive effects of cannabis, while cannabidiol does not induce psychoactive effects and can have a protective effect.
- The effects induced after cannabis abuse will depend on the concentrations of Δ^9-THC and cannabidiol.
- As of this writing, three different cannabinoid drugs have been approved with different indications.
- Research on the development of various cannabinoid drugs is very active, following different strategies: targeting peripheral cannabinoid receptors, receptors expressed in a certain tissue, or cannabinoids combined with other drugs.

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