Review

Psychopharmacology of the endocannabinoids: far beyond anandamide

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Abstract

The study of endocannabinoid pharmacology has proceeded from the discovery of Δ9-tetrahydrocannabinol, the main psychoactive compound in *Cannabis sativa*, to the identification of an endogenous endocannabinoid system that is essential for physiological modulation of neuronal functions. We have not yet achieved a complete understanding of the various roles of the endocannabinoids, but this is one of the fastest-growing fields in psychopharmacology. This review starts with a brief historical description of the discovery of the endocannabinoids and then focuses on recent pharmacological advances and recently discovered endocannabinoid mechanisms of action (e.g. functional selectivity, allosterism, and receptor trafficking). Finally, we will discuss the contention that the existence of evidence-based therapeutic applications for cannabinoids and the wide range of physiological functions affected by endocannabinoids suggests that the careful study of the endocannabinoid system may lead to the development of novel therapeutic drugs with higher societal acceptability and lower side effects profiles.

Keywords

2-arachidonoyl-glycerol, allosterism, anandamide, **c**annabis, cannabinoid, CB1, CB2, endocannabinoid, functional selectivity, GPR55, N-arachidonoyl-dopamine, N-arachidonoyl-glycine, noladin, receptor trafficking, TRPV1, virodamine

Introduction

Recent decades have witnessed a true revolution in our understanding of the endocannabinoid system and its importance for neuronal transmission. Extensive studies of endocannabinoid physiology and pharmacology have brought us from an initial acknowledgment that the main psychoactive compounds of *Cannabis sativa* bind to specific sites in the brain to the actual discovery of two cannabinoid receptors, two to five endogenous ligands, and a number of endocannabinoid metabolic enzymes. We have not yet achieved a complete understanding of the various roles of endocannabinoids, but this is one of the fastest-growing fields in psychopharmacology. As shown in Figure 1, the number of published psychopharmacology articles that contain the term 'endocannabinoid' in the title, the abstract, or both, has dramatically increased in the last decade (2000–2010). In the same period, the number of published studies addressing 'classic' neurotransmitters has declined or remained constant.

The term endocannabinoid was 'officially' coined in articles from the mid-1990s authored by the Italian researcher Vincenzo di Marzo (Di Marzo and Fontana, 1995; Di Marzo et al., 1994) and has since become increasingly popular. Because of the increasing importance of the endocannabinoids in the international scientific literature covering the field of psychopharmacology, the objectives of the present review are to examine the scientific data generated in the last few decades, attempt to provide the reader with a historical context to this controversial research field, and describe an updated overview of endocannabinoid psychopharmacology. To these ends, we first revisit the ancient relationship between humanity and the *Cannabis* plant, and then examine how multidisciplinary scientific investigations have resulted in the discovery of the endocannabinoid system. Next, we will scrutinize what is currently known about the biochemistry and pharmacology of the endocannabinoids. Finally, we will conclude by discussing the recent studies of putative novel endogenous ligands that target this system and suggest future directions for cannabinoid research.

The controversial use of *Cannabis* from ancient to modern times

Derivatives of the *Cannabis* plant are among the earliest plants cultivated by humankind. Historical and archaeological findings indicate that *Cannabis* has been cultivated in

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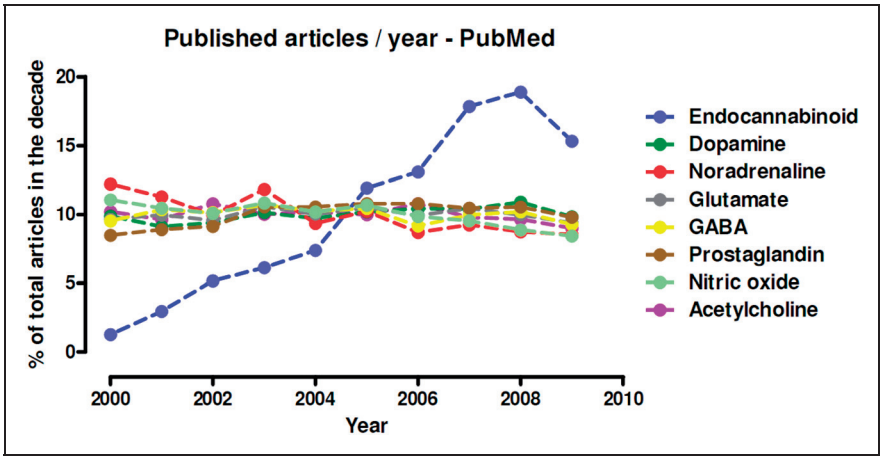

Figure 1. The number of psychopharmacology studies published using the term endocannabinoid has increased dramatically in the last decade. Articles were included in the analysis when the name of each of the neurotransmitters listed in the figure legend and the word brain were present in the title, the abstract, or both parts of the article according to the PubMed search engine (http://www.ncbi.nlm.nih.gov/pubmed/). The graph illustrates the percentage of articles including those terms published in each year relative to the total number of articles including those terms published in the entire decade (2000–2010).

China since 4000 BC (Li, 1973), and the psychopharmacological use of *Cannabis* was reported in the Chinese pharmacopoeia that dates back to 2700 BC (Li, 1973). A recent exhumation of a 2700-year-old grave revealed a large quantity (789 g) of *Cannabis* that still contained psychoactive cannabinoids (Russo et al., 2008). Interestingly, the size of the *Cannabis* seeds, the color and morphology of the *Cannabis*, and the large amount of processed (pounded) *Cannabis* all suggested that the sample was cultivated and harvested rather than merely gathered from wild plants (Russo et al., 2008). In India, since 1000 BC, *Cannabis* had a strong association with religious practices; the sacred Vedas texts refer to it as '*a source of happiness, donator of joy, and bringer of freedom*' (Touwn, 1981). Medical use of *Cannabis* in India included its use as analgesic, anticonvulsant, hypnotic, tranquilizer, anesthetic, anti-inflammatory, antibiotic, antiparasitic, antispasmodic, pro-digestive, appetite stimulant, diuretic, aphrodisiac, antitussive, and expectorant (Touwn, 1981). Some of these uses have been supported by contemporary medicine (Consroe, 1998; Di Marzo and Petrocellis, 2006; Hollister, 1986); other uses are still a matter of debate, often because of political and societal influences rather than medical or scientific data (Grotenhermen, 2007).

The introduction of *Cannabis* in Western medicine occurred in the first half of the 19th century, through the studies of the Irish physician William B O'Shaughnessy and the French psychiatrist Jacques-Joseph Moreau (Zuardi, 2006). O'Shaughnessy first encountered *Cannabis* in India, and he began to study several preparations of this plant in animals and humans (Di Marzo, 2006). Moreau first encountered *Cannabis* with Arabs, and he decided to systematically experiment on himself and his students with different preparations. Clearly impressed by the effects of the plant, Moreau declared that hashish was '*a powerful and unique method to investigate the genesis of mental illnesses*' (Moreau, 1845). The work of these two men spread the medicinal use of *Cannabis* from England and France to the rest of Europe, and then later to North America. By the second half of the 19th century, the scientific community had acknowledged the therapeutic value of *Cannabis*, and various laboratories marketed *Cannabis* extracts or tinctures as conventional medicines (Fankhauser, 2002). For a comprehensive review of the history of *Cannabis* as a therapeutic drug, we recommend the review by Zuardi (2006).

In Europe, the use of *Cannabis* was popular among intellectuals, who gathered in small groups (called hashish clubs) to smoke the plant resin (19th century). In the Americas, the use of *Cannabis* was probably introduced by African slaves and became relatively common in the rural areas of northeastern Brazil from the 16th century. Four hundred years later, the use of *Cannabis* in Brazil remained restricted to small low-income groups, and it was known as the 'opium of the poor' (Pinho, 1975). *Cannabis* was also used in the most underprivileged populations of Mexico, and immigrants from these communities introduced the recreational use of *Cannabis* to the USA. In that country, use of *Cannabis* remained restricted to Black and Hispanic immigrant neighborhoods until the middle of the 20th century (Musto, 1972).

Despite its ancient tradition, modern history imposed many legal restrictions on the medical, recreational, and religious use of *Cannabis*. In the USA, the Marihuana Tax Act law (1937) introduced taxes and other difficulties for *Cannabis* users; in 1970, the Controlled Substances Act made possession of marijuana illegal in the USA, with repercussions throughout the world (Zuardi, 2006). Accordingly, the number of scientific studies regarding *Cannabis* tended to decline during the following two decades. Recently, it has become clear that prohibition has had only moderate success in reducing the prevalence of *Cannabis* use. At the same time, prohibition has hampered the benefits to people and society that would arise from the development of therapeutic uses for

Cannabis, including for people suffering from severe illnesses, such as multiple sclerosis or chronic neuropathic pain (Pryce and Baker, 2005; Rahn and Hohmann, 2009). For a recent review of *Cannabis* prohibition see Grotenhermen (2007).

A brief history of the discovery of the endocannabinoid system

Despite the long history of the human relationship with the *Cannabis* plant, knowledge regarding the phytochemistry and pharmacology of cannabinoids is primarily derived from studies conducted in the last 50 years. The major active chemical in *Cannabis*, Δ^9-tetrahydrocannabinol (Δ^9-THC), was isolated from the plant, molecularly characterized, and synthesized in the laboratory of Raphael Mechoulam in the mid 1960s (Gaoni and Mechoulam, 1964). While the identification of Δ^9-THC did not fully explain the biological effects of *Cannabis* in humans, this finding dramatically increased scientific research in this field, and approximately 60 other cannabinoids have been identified in *Cannabis* flowers (Mechoulam and Hanus, 2000). During that time, Billy Martin developed the cannabinoid tetrad test, which consists of the simultaneous observation of four typical cannabinoid effects (hypolocomotion, analgesia, hypothermia, and catalepsy) and is still used today for the behavioral screening of cannabinoid compounds (Martin, 1985). The *in vivo* characterization of Δ^9-THC was followed by several initial *in vitro* tests using membranes of neuronal tissues and radiolabeled Δ^9-THC. These tests confirmed the biological effects of this molecule and linked them to activation of G proteins, but they did not identify a specific site of action (Howlett, 1987), because the lipophilicity of Δ^9-THC leads to high non-specific membrane binding (Roth and Williams, 1979). The radiolabeled synthetic cannabinoid agonist CP-55,940 supported the identification of the specific binding sites of Δ^9-THC in the brain (Devane et al., 1988). The definitive cloning and elucidation of the molecular structure of CB1 receptors has been conducted from a list of orphan metabotropic receptors (Matsuda et al., 1990), and was promptly followed by the identification of the CB2 receptor through structural analogy (Munro et al., 1993). Recent evidence suggests that a number of other orphan receptors such as GPR55 (Ryberg et al., 2007), GPR18 (Kohno et al., 2006), and GPR119 (Overton et al., 2006) may constitute new types of cannabinoid receptors.

A few years after the discovery of CB1 and CB2, the first endogenous molecule binding to these receptors was extracted from the porcine brain, suggesting the existence of the endocannabinoid system (Devane et al., 1992). This endocannabinoid agonist is the ethanolamine of arachidonic acid and was named anandamide (AEA), whose name was inspired by the Sanskrit word ananda (meaning 'overjoy' or 'bliss'). A second endocannabinoid 2-arachidonoyl-glycerol (2-AG) was identified a few years later by two independent research groups (Mechoulam et al., 1995; Sugiura et al., 1995). Other lipid molecules, such as oleamide (Leggett et al., 2004), O-arachidonoyl etanolamine (virodamine) (Porter et al., 2002), 2-arachidonoyl glyceryl ether (noladin) (Hanus et al., 2001), and the N-arachidonoyl-dopamine (NADA) (Bisogno et al, 2005; Huang et al, 2001) have also been suggested to exert cannabimimetic activity.

Figure 2 illustrates recent developments in cannabinoid research. We show independent curves representing the absolute number of publications listed in the PubMed database with the terms 'cannabis', 'cannabinoid' or 'endocannabinoid' in the title, the abstract, or both. Note the inflection point caused by the discovery of cannabinoid receptors and ligands at the beginning of the 1990s (Figure 2). From 2000 to 2010, roughly 46% of the papers in this field were devoted to the study of the cannabinoids, 31% to the study of *Cannabis*, and 23% to the study of the endocannabinoids. This historical trend suggests that the endocannabinoids will continue to gain in importance, especially since various details of the physiological functions and therapeutic potentials of endocannabinoids have not yet been characterized (Bellocchio et al., 2006; Kunos et al., 2009; Mackie, 2006; Pertwee, 2005; Schneider et al., 2005; Steffens and Mach, 2006).

Brain and neuronal distribution of cannabinoid receptors

Initial autoradiography studies using the radiolabeled cannabinoid agonist [^{3}H]CP-55,940 revealed that the expression of CB1 receptors in the brain is extremely high, and it is comparable with the expression of ionotropic GABA and glutamate receptors (Herkenham et al., 1990). Indeed, today these receptors are considered the most abundant metabotropic receptors in the mammalian brain. Moreover, the regional distribution of the cannabinoid receptors is consistent with the characteristic psychoactive effects of Δ^9-THC (Howlett et al., 2002). For instance, CB1 receptors are highly expressed in brain areas related to movement control, such as the basal ganglia and cerebellum (a neural substrate for hypolocomotion and catalepsy), and in corticolimbic areas related to the regulation of emotional and cognitive processes, including the cingulate cortex, frontal cortex, amygdala, and hippocampus. Moderate expression levels are observed in the dorsal root of the spinal cord, the periaqueductal gray matter, cortical areas related to nociceptive processing (neural substrates for analgesia), and the hypothalamus (a neural substrate for neuroendocrine effects). Relatively low levels of CB1 receptors are expressed in areas involved in the control of essential vegetative functions (Herkenham et al., 1990).

More detailed *in situ* hybridization studies have shown that both excitatory and inhibitory synapses may contain CB1 receptors with distinct expression patterns. Although GABAergic inhibitory interneurons contain high levels of mRNA for CB1 receptors in punctuated patterns (Bodor et al., 2005; Marsicano and Lutz, 1999), excitatory glutamatergic neurons contain lower levels of CB1 expression with granulated aspects (Domenici et al., 2006; Kawamura et al., 2006; Marsicano and Lutz, 1999; Monory et al., 2006). In the forebrain, the high and punctuated expression of CB1 receptors is associated with a subgroup of GABAergic interneurons expressing cholecystokinin (CCK), but it is not associated with GABAergic interneurons expressing parvalbumin (Bodor et al., 2005; Hajos et al., 2000; Katona et al.,

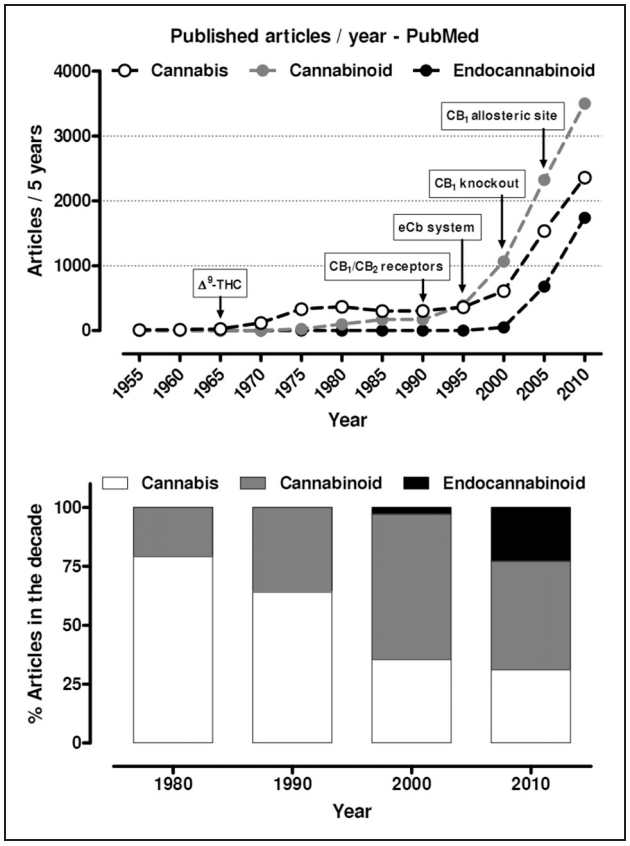

Figure 2. Important hallmarks in the history of research on *Cannabis*, cannabinoids, and the endocannabinoid system. *Top:* The number of psychopharmacology studies published using the terms *Cannabis*, cannabinoid or endocannabinoid since 1950, binned by each 5-year period. Note the increase in *Cannabis* research after the discovery of Δ^9-THC, the main psychoactive cannabinoid found in *Cannabis*. Furthermore, note the increase in cannabinoid research following the identification of the CB_1/CB_2 cannabinoid receptors and the start of endocannabinoid research by the mid-1990s. *Bottom:* Scientific interest is shifting from *Cannabis* research to endocannabinoid research. The percentage of articles published using each individual term relative to the total number of articles using any of the three terms published in each decade from 1980 to 2010 suggests a shift from *Cannabis* research to cannabinoid research in the decades of 1990 and 2000 and more recently towards endocannabinoid research. The search only included articles where each of the terms listed in the figure legend and the word brain were present in the title, the abstract, or both, according to the PubMed search engine (http://www.ncbi.nlm.nih.gov/pubmed/).

1999). Despite this, in the striatum, CB1 receptors are also expressed in parvalbumin-positive interneurons (Marsicano and Lutz, 1999). Moreover, in certain hypothalamic nuclei, CB1 receptors are expressed only in principal glutamatergic neurons. These findings illustrate the complex variability in the expression of CB1 receptors, which depends on the cell type and brain region (Marsicano and Lutz, 1999). At a subcellular level, the majority of CB1 receptors are localized to the pre-synaptic terminals of neuronal axons, and low levels of these receptors are found in proximal axonal regions, dendrites, or the cell body (Leterrier et al., 2006; Nyiri et al., 2005).

For many years, there was considered to be a clear division in the distribution of cannabinoid receptors. It was believed that CB1 receptors belonged primarily to neuronal tissues (but were also found in other tissue types), whereas CB2 receptors were expressed exclusively in peripheral tissues and immune cells (Howlett et al., 2002). Consequently, CB1 receptors were nicknamed the 'neuronal cannabinoid receptors', whereas the CB2 receptors were nicknamed the 'peripheral cannabinoid receptors'. However, some researchers accepted that CB2 receptors could at least be expressed in glial cells (Buckley et al., 1998; Galiegue et al., 1995). This dichotomy persisted until the pioneering studies of Marja Van Sickle and Emmanuel Onaivi, who reported the presence of functional CB2 receptors in the brain stem, cortex, and cerebellum of rats (Van Sickle et al., 2005), and the later confirmation of these reports by modern molecular biology techniques (Onaivi et al., 2006). Moreover, the development of a new antibody allowed for the identification of the presence of CB2 receptors in other brain regions – such as the striatum, hypothalamus, cortex, substantia nigra, amygdala, and hippocampus – and that these receptors were absent in CB2 knockout mice (Onaivi et al., 2006). According to these authors, the expression of CB2 receptors in the brain stem is about 100 times lower than the expression of CB1 receptors. Importantly, the expression of CB2 mRNA in the brain is approximately 1–2% of the established expression levels found in the spleen. These relatively low expression levels may explain why central CB2 receptors were previously missed. Furthermore, immunoelectron microscopy has allowed the elucidation of the ultrastructural localization of CB2 receptors in neurons. In sharp contrast to CB1 receptors, CB2 receptors are mainly expressed in post-synaptic dendritic processes and in the cell body (Onaivi et al., 2006).

The residual effects of the synthetic cannabinoid agonist WIN55,212-2, the endogenous agonist AEA, and CB1 antagonists in CB1 receptor knockout mice suggest the existence of at least a third type of cannabinoid receptor (Di Marzo et al., 2000; Prather et al., 2000). This receptor would be sensitive to the antagonist SR141716A (rimonabant) and insensitive to the antagonist AM251 (Begg et al., 2005; Brown, 2007). Nevertheless, the putative CB3 receptor has not yet been reliably characterized. This and other evidence of new cannabinoid receptors will be addressed later in this review.

The endocannabinoids, synthesis and degradation

AEA and 2-arachidonoylglicerol (2-AG) were identified as the first endocannabinoids shortly after the cloning of the CB1 and CB2 cannabinoid receptors. Over the subsequent years, several other lipids were suggested as candidate endocannabinoids, mainly because two receptors and two endogenous ligands seemed to be an insufficient set of elements to explain the diversity and complexity of neuronal modulation by the endocannabinoid system. Figure 3 provides an updated view of this subject, listing the two widely accepted endocannabinoids, AEA and 2-AG, and three other putative

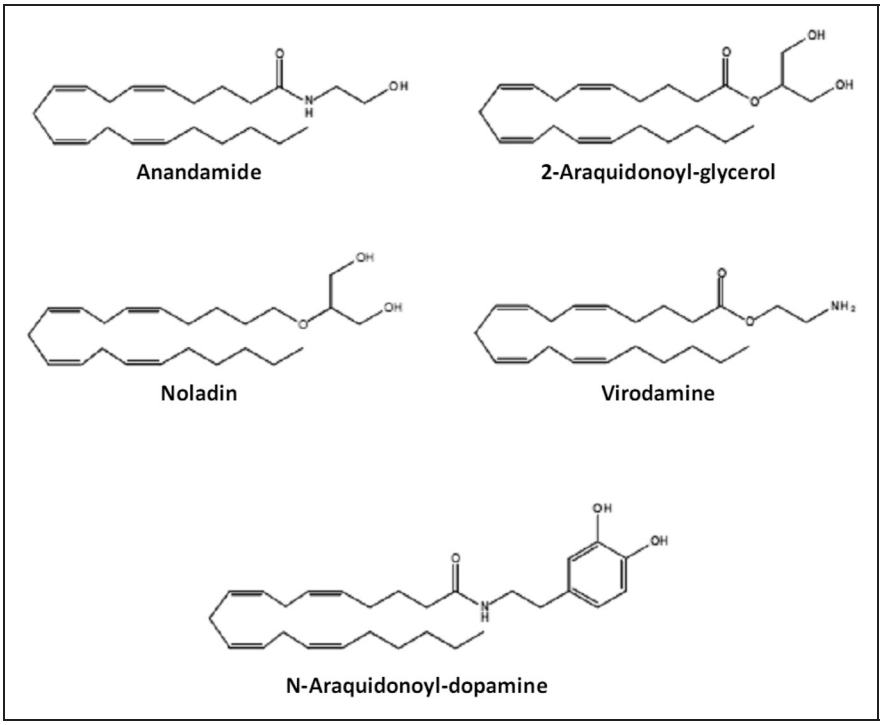

Figure 3. Schematic representation of the molecular structures of the two most widely accepted endocannabinoids (Anandamide and 2-Arachidonoyl-glycerol) and three other endogenous cannabimimetic molecules that have thus far been identified. Other candidate molecules are being investigated, and the actual number of endocannabinoids may be more than eight.

endocannabinoids. A remarkable aspect of the endocannabinoids is that they are not stored in resting cells but are synthesized and released on demand following stimulation (Di Marzo and Deutsch, 1998). A common structural feature is their lipophilic nature, being constituted by a polyunsaturated fatty acid moiety (arachidonate) and a polar head group, ethanolamine (in the case of AEA) or glycerol (in the case of 2-AG). There is not yet full agreement on the topic, but here we briefly list the most widely accepted model for the synthesis of AEA and 2-AG, which are likely produced by distinct, though not entirely independent, biosynthetic routes.

A general rule for the biosynthetic route of endocannabinoids is that they are synthesized from membrane phospholipids that are hydrolyzed via phospholipase pathways to the final neurotransmitter. AEA is formed from the precursor N-arachidonoylphosphatidylethanolamine (NAPE). NAPE is produced by N-acyltransferase when it transfers a arachidonate group from phospholipids to the primary amino group of phosphatidylethanolamine (Di Marzo et al., 1994). NAPE is subsequently hydrolyzed to AEA via NAPE-PLD (Di Marzo et al., 1994; Sugiura et al., 1996). Cyclic AMP and Ca^{+2} can modulate N-acyltransferase activity and thereby control the amount of substrate available for AEA synthesis (Cadas et al., 1996). In addition, activation of dopamine D2, muscarinic M1/M3, and glutamate mGluR1 metabotropic receptors can trigger AEA synthesis (Giuffrida et al., 1999; Kim et al., 2002; Varma et al., 2001). The synthesis of 2-AG may occur via two redundant synthetic routes, likely reflecting its involvement in cellular metabolism, in addition to its role as a neurotransmitter (Stella et al., 1997; Sugiura et al., 1995). One of the synthetic pathways for 2-AG involves the second messenger diacylglycerol (DAG), which is produced by the hydrolysis of membrane phospholipids via a phospholipase C-dependent mechanism. Then, DAG is converted to 2-AG via a Ca^{+2}-dependent DAG lipase (Farooqui et al., 1989). Accordingly, a common feature of all endocannabinoids' synthetic routes is their dependence on increases of intracellular Ca^{+2} (Stella et al., 1997).

The existence of different routes for AEA and 2-AG synthesis suggests that these two endocannabinoids operate independently. Although this may be true in a number of cases (Ferrer et al., 2003; Giuffrida et al., 1999; Stella and Piomelli, 2001; Stella et al., 1997), there is recent evidence of a shared mechanism of biosynthesis between these endocannabinoids (Maccarrone et al., 2008). As the precursors and synthetic enzymes for endocannabinoids are on the cell surface, it seems reasonable that the endocannabinoids are generated in the plasma membrane and released by diffusion, either passive or facilitated by lipid-binding proteins (Piomelli, 2003). The action of endocannabinoids is essentially terminated by a reuptake system that is present in both neurons and glia. However, the protein mediating this reuptake has not yet been identified (Beltramo et al., 1997; Hillard et al., 1997). There is compelling evidence that endocannabinoid reuptake is selective, saturable, temperature dependent, sensitive to pharmacological inhibition, and entirely shared by AEA and 2-AG, which suggests the existence of a protein that

functions as an endocannabinoid transporter (Beltramo and Piomelli, 2000; Beltramo et al., 1997; Bisogno et al., 2001; Di Marzo et al., 1994; Hillard et al., 1997). Notably, several families of fast and selective lipid transporters have already been characterized (Abumrad et al., 1999; Hirsch et al., 1998; Schaffer and Lodish, 1994).

Once taken up by cells, AEA is mainly metabolized to arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH) and 2-AG is mainly metabolized to arachidonic acid and glycerol by monoacylglycerol lipase (MGL), but also to some extent by FAAH (Cravatt et al., 1996, 2001; Dinh et al., 2002). FAAH and MGL are widely expressed in the brain, but while FAAH is expressed in postsynaptic structures, MGL is mostly associated with nerve endings (Cravatt et al., 1996). There is also evidence of oxidative endocannabinoid metabolism by cyclooxygenases (COX), lipoxygenases (LOX), and P450 cytochromes (Kozak and Marnett, 2002; van der Stelt et al., 2002), suggesting an interaction between the different metabolic pathways of eicosanoids.

COX-2 is responsible for the oxidation of AEA and 2-AG to oxygenated derivatives of prostaglandins, namely prostaglandin ethanolamides (prostamides) and prostaglandin glycerol esters (Woodward et al., 2008). This oxidative pathway seems to be primarily an inactivation pathway, since the oxidation of AEA to PGE2-EA, PGA2-EA, and PGB2-EA dramatically reduces its binding to CB1 receptors (Pinto et al., 1994). AEA and 2-AG are metabolized by purified COX-2 in high substrate concentrations ($>100\,\mu M$ *in vitro*) and with low affinity ($Km = 24–60\,\mu M$ *in vitro*), and they are not metabolized by COX-1 (Kozak et al., 2000, 2001;Yu et al., 1997). 2-AG oxidation usually leads to production of PGH2-G and HETE-G, two lipids with no known biological activity (Kozak et al., 2000, 2001).

LOX-mediated metabolism generates structural derivatives with substantial biological activity (Craib et al., 2001; Edgemond et al., 1998; Kozak and Marnett, 2002). The LOX enzymes show a reasonable affinity for the endocannabinoids, with the following order of oxygenase activity: 15-LOX > 12-LOX > 5-LOX, the last being practically inactive (Ueda et al., 1995). The product of AEA metabolism by 12-LOX retains its affinity for the CB1 receptors. In contrast, the product of 15-LOX metabolism does not bind to CB1 receptors, but does inhibit FAAH (Edgemond et al., 1998; van der Stelt et al., 2002). Other hydroxylated derivatives of AEA are suggested to act via TRPV1 (Craib et al., 2001). Indeed, recent structural studies have shown that 12-LOX is expressed in neurons in close proximity to TRPV1 receptors, and because of the high cellular colocalization between vanilloid and cannabinoid receptors, 12-LOX may also be expressed in close proximity to CB1 receptors (Cristino et al., 2008). LOX-mediated metabolism of endocannabinoids may have cell-type-dependent biological relevance. For example, in platelets, where the expression of FAAH and COX-2 is negligible, endocannabinoid metabolism may occur primarily via LOX enzymes (Kozak and Marnett, 2002). As another example, the metabolism of 2-AG by 15-LOX generates a derivative that activates PPARα; receptors. Finally, 2-AG is a preferential substrate for 12-LOX in leucocytes, and it is reasonable to wonder whether a similar phenomenon may occur in other cell types, such as in the central nervous system (Kozak and Marnett, 2002).

The metabolism of endocannabinoids by P450 cytochromes has been rarely studied, but non-oxygenated AEA derivatives may be generated through this pathway, but with still undefined biological activity (Bornheim et al., 1995). At least 20 different AEA products may be generated in reactions mediated by P450 cytochromes, including epoxidation, ω-hydroxylation, lipoxigenation, and oxidation (Bornheim et al., 1993; Capdevila and Falck, 2001).

Most studies of endocannabinoid metabolism have examined pathways generating structural modifications of the arachidonoyl moiety of these lipids. However, both AEA and 2-AG appear to have hydrophilic sites of oxidative metabolism. The investigation of this possibility resulted in the discovery of a polar derivative of AEA named N-arachidonoylglycine (Burstein et al., 2000). This lipoamino acid seems to be formed by the condensation of arachidonic acid and glycine (Huang et al., 2001). Lipoamino acids appear to be physiologically relevant and may be therapeutically promising, as N-arachidonoyl-glycine is a potent antinociceptive agent (Burstein et al., 2000; Huang et al., 2001). It is important to note, however, that N-arachidonoylglycine has practically no affinity for CB1 (Sheskin et al., 1997) or TRPV1 receptors (Huang et al., 2001). As such, the mechanism of action of the antinociceptive effects of lipoamino acids remains to be elucidated. Figure 4 summarizes our current understanding of AEA metabolic pathways.

Little is known about the anabolic or catabolic enzymatic pathways for other endocannabinoids. Investigations of the interactions between the eicosanoid pathways are only now beginning. Because a substantial part of the work that has been completed was done *in vitro*, the physiological relevance of such findings is yet to be established. Nevertheless, an intriguing possibility has already emerged, as it appears that some oxidative pathways may contribute not only to endocannabinoid inactivation but also to the generation of biologically active derivatives. Henceforth, the biotransformation of cannabinoids has to be considered in a qualitative fashion, and we must acknowledge that certain organs, tissues, or cell types may represent special biochemical environments with specific endocannabinoid effects.

Endocannabinoid pharmacology

The cannabinoid receptors have a similar primary signaling system. Both CB1 and CB2 receptors are coupled to $G_{i/o}$ proteins and act through adenylyl cyclase inhibition, with consequential reductions of cAMP levels, inhibition of voltage-dependent Ca^{+2} channels (L, N, P, Q types), and activation of K^+ channels (A type). Cannabinoid receptors also activate MAP kinase and PI3 kinase pathways among other intracellular routes (Howlett et al., 2004; Pacher et al., 2006). The functional outcome of receptor activation is the suppression of neuronal excitability and inhibition of depolarization-induced neurotransmitter release, including monoamines, amino acids, and neuropeptides (Howlett et al., 2002). Despite this general picture, cannabinoid effects vary quantitatively and qualitatively depending on the tissue and the cell type. For example, when CB1 receptors are expressed in

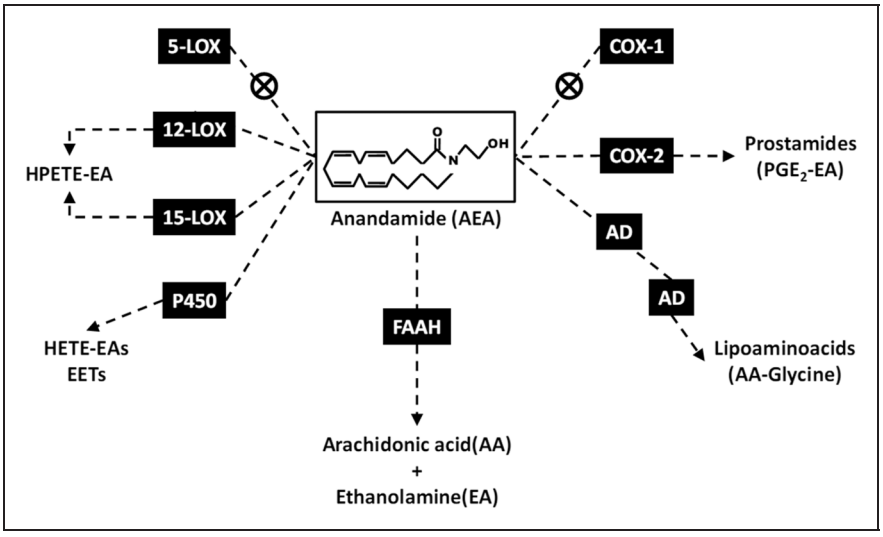

Figure 4. Enzymatic metabolic pathways for anandamide (AEA) catabolism. The main catabolic pathway for AEA degradation uses fatty acid amide hydrolase (FAAH) and generates both arachidonic acid (AA) and ethanolamine (EA). Alternative routes use the 12- or 15-lipoxygenase (LOX) pathways that generate hydroperoxide-eicosatetranoyl-etanolamide (HPETE-EA), the cyclooxygenase 2 (COX-2) pathways that generate of prostamides such as PGE2-EA, the P450 cytochrome pathway that generates hydroxy-ethanolamine eicosatrienoic acid (HPETE-EA) and epoxy-eicosatrienoic acid (EET), and the sequential pathways of alcohol and aldehyde dehydrogenase (AD) that generate lipoamino acids such as arachidonoyl-glycine (AA-Glycine).

Table 1. Target receptors for endocannabinoids

Endocannabinoid	CB_1	CB_2	GPR55	$TRPV_1$	PPARα	PPARδ
AEA	++	++	++	++	++	++
2-AG	+++	+++	++	0	0	0
Noladin	++	+	++	0	++	0
Virodamina	–	++	+++	0	++	0
NADA	++	0	0	+++	0	0

Activity of endocannabinoids at metabotropic receptors (CB_1, CB_2, and GPR55), ligand-activated ionic channels (TRPV1), and nuclear receptors (PPARα, e, and δ). Legend: 0 no activity, – antagonist, + weak partial agonist, ++ partial agonist, +++ full agonist. AEA, anandamide; 2-AG, 2-arachydonoyl glycerol; NADA, n-araquidonoyl dopamine (adapted from Alexander SPH and Kendall DA (2007) The complications off promiscuity: endocannabinoid action and metabolism. Br J Pharmacol 152: 602–623 with permission from John Wiley & Sons).

inhibitory interneurons (as they often are), the net effect of CB1-dependent inhibition of inhibitory interneuron release of GABA is a disinhibition that indirectly increases downstream *in vivo* neurotransmitter release. Furthermore, direct CB1-mediated excitatory effects have been described in artificial *in vitro* settings wherein coupling of inhibitory G proteins is pharmacologically blocked. Surprisingly, under these conditions, CB1 receptors can signal via excitatory G_s proteins and stimulate cyclic AMP formation (Glass and Felder, 1997; Rhee et al., 1998).

Endocannabinoids can also bind to non-CB1 receptors. For instance, AEA activates vanilloid $TRPV_1$ receptors (Starowicz et al., 2007) and PPAR receptors for lipids (O'Sullivan, 2007). Moreover, recent studies indicate that endocannabinoids activate the previously orphaned receptor GPR55 (Ryberg et al., 2007). The next paragraphs will describe the interactions among endocannabinoids and these receptors. The pharmacology of endocannabinoids is summarized in Table 1.

Although they present low structural homology with CB1 and CB2 receptors, GPR55 receptors have high affinity binding sites for endocannabinoids, synthetic cannabinoids, and CB1 antagonists (Brown, 2007). The synthetic cannabinoid agonist WIN55,212-2 does not bind to GPR55 receptors, which precludes the possibility that GPR55 is the purported CB3 receptor (Brown, 2007). Nevertheless, GPR55 may be the receptor-binding target of the phytocannabinoid cannabidiol, which has been suggested to be neither the CB1 nor the CB2 receptor. GPR55 receptors are expressed in peripheral organs, such as the spleen (similar to CB2 receptors), and in many brain regions, including the hippocampus, thalamus, frontal cortex, cerebellum, striatum, hypothalamus, and brain stem. GPR55 receptors do not signal via $G_{i/o}$ proteins; instead, they couple with G_{13} and indirectly signal via monomeric small G proteins of the Ras family (Ryberg et al., 2007).

PPAR receptors belong to a family of nuclear receptors, which have three isoforms (i.e. α, Δ, and γ). These receptors form heterodimers with retinoid X receptors and bind to

PPAR-responsive elements in the DNA, thereby promoting transcription of target genes upon ligand activation (O'Sullivan, 2007). PPARs target genes involved in regulation of metabolism and energy homeostasis, cell differentiation, and inflammation (Ferre, 2004). In addition to the direct binding of endocannabinoids to PPAR receptors, some evidence suggests that the activation of PPAR by other ligands regulates CB1 receptor expression. As many other fatty acids derivatives interact with PPAR receptors, it is not totally surprising that these receptors take part in endocannabinoid signaling (O'Sullivan, 2007).

TRPV1 vanilloid receptors are Ca^{2+}-permeable non-selective ionic channels that have been primarily found in sensory neurons from dorsal root ganglia. Recently, these receptors have been identified in brain regions, including the hippocampus and periaqueductal gray matter. Indeed, it has been suggested that, under certain conditions, some endocannabinoid molecules such as AEA have higher efficacy at TRPV1 than CB1 receptors (Di Marzo et al., 2001). Because of this, AEA is sometimes considered an endovanilloid (Starowicz et al., 2007). An alternative view is that there is an intense exchange between the endocannabinoid and endovanilloid systems. However, to the best of our knowledge, selective endogenous ligands of TRPV1 have not yet been convincingly characterized to assume the existence of an endovanilloid system. Lipoxygenase-derived eicosanoids are candidate ligands as selective endovanilloids, such as 12-HPETE (Cristino et al., 2008; Shin et al., 2002).

Other lipid mediators, despite showing very low or even no affinity for known cannabinoid receptors, still present cannabimimetic activity. For example, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) are two lipids that share structural similarity with AEA, induce analgesia, and modulate food intake, even though they apparently do not bind to cannabinoid receptors (Lambert and Di Marzo, 1999; Lambert et al., 1999; Rodriguez de Fonseca et al., 2001). Evidence suggests that OEA may bind to GPR119 and PEA may bind to GPR55, two formerly orphaned receptors that have been increasingly considered as candidate cannabinoid receptors (Fu et al., 2005; LoVerme et al., 2005; Mackie and Stella, 2006; Overton et al., 2006). The lipoamino acid N-arachidonoylglycine exerts AEA-like analgesic effects, but it is devoid of other typical cannabinoid effects (Huang et al., 2001), has no affinity for CB1 receptors (Sheskin et al., 1997), does not alter endocannabinoid uptake (Huang et al., 2001), and does not bind to vanilloid TRPV1 receptors (Huang et al., 2001). However, it does inhibit AEA metabolism by FAAH (Huang et al., 2001) and thereby elevates the *in vivo* levels of AEA (Burstein et al., 2002). Moreover, it seems that N-arachidonoylglycine activates the orphan receptor GPR18 (Kohno et al., 2006; Samuelson et al., 1996). Other lipoamino acids such as N-arachidonoylserine have also been found in the brain and in peripheral tissues (Bradshaw and Walker, 2005).

In addition to these main classes of cannabinoid and non-cannabinoid receptors, AEA reduces the conductances of α; 7 nicotinic receptors, glycine receptors, and $5HT_3$ serotonergic receptors. In addition, there are a number of non-receptor targets for endocannabinoids that have been described (for an excellent review see Oz, 2006). Yet, a major challenge in this field is to determine which interactions are physiologically relevant and which occur merely because of molecular interactions in the artificial environment of *in vitro* assays, and are therefore not relevant to *in vivo* endocannabinoid release.

Future directions for endocannabinoid research

The preceding sections reviewed the history of cannabinoid research, traced the scientific route that led to the discovery of the endocannabinoid system, and briefly described several different elements of the biochemistry and pharmacology of this intriguing neuromodulatory system (illustrated in Figure 5). To conclude, we will discuss up-to-date information on aspects of endocannabinoid pharmacology that constitute new research opportunities for the scientific community. Specifically, we will discuss the concept of endocannabinoid functional selectivity, allosteric modulation of CB1 receptors by ligands, receptor dimerization, or both, and the impact of CB1 receptor trafficking on cannabinoid functions (Figure 6). The traditional understanding of metabotropic receptors as mere on/off switches of a single transduction system has been replaced by the view that G-protein coupled receptors are versatile and dynamic molecules that 'adapt' to ligands rather than being statically 'selected' by them. In this conceptualization, the cellular environment plays an essential role in receptor pharmacology (Kenakin, 2007). It has been suggested that there are microdomains in the cellular membrane with specialized lipid compositions, known as caveolae and lipid rafts. Furthermore, these microdomains may have important roles in endocannabinoid transmission, such as influencing the activity of some endocannabinoid synthetic enzymes (Placzek et al., 2008), modulating cellular reuptake of endocannabinoids (McFarland et al., 2004), and regulating the affinity of CB1 receptors for endocannabinoids (Bari et al., 2005).

The traditional conceptualization of CB1 pharmacology would suggest that 'these are inhibitory receptors that act through activation of $G_{i/o}$ proteins, leading to reduction of neuronal excitability and inhibition of neurotransmitter release'. This classical view has been challenged by studies showing novel coupling of CB1 receptors. Direct CB1-mediated 'excitatory' effects have been described in artificial *in vitro* settings in which coupling with inhibitory $G_{i/o}$ proteins is pharmacologically blocked by pertussis toxin. This demonstrates that the assumption that CB1 receptors are pre-synaptic receptors devoted to the inhibition of neurotransmitter release may be an oversimplification. CB1 receptors can, surprisingly, signal via excitatory G_s proteins, stimulate adenylate cyclase, and increase the formation of the second messenger cAMP (Glass and Felder, 1997; Rhee et al., 1998). Furthermore, successive activation of G_s and $G_{i/o}$ proteins by increasing concentrations of cannabinoid agonists leads to biphasic concentration–response profiles, such as biphasic regulation of voltage-gated Ca^{2+} channels (Fan and Yazulla, 2003; Rubovitch et al., 2002) or GABA release (Gonzalez et al., 2009). Tolerance to cannabinoid effects may result from a switch from $G_{i/o}$ to G_s coupling by CB1 receptors (Paquette et al., 2007). Physiological activation of different unrelated G proteins provides a complex

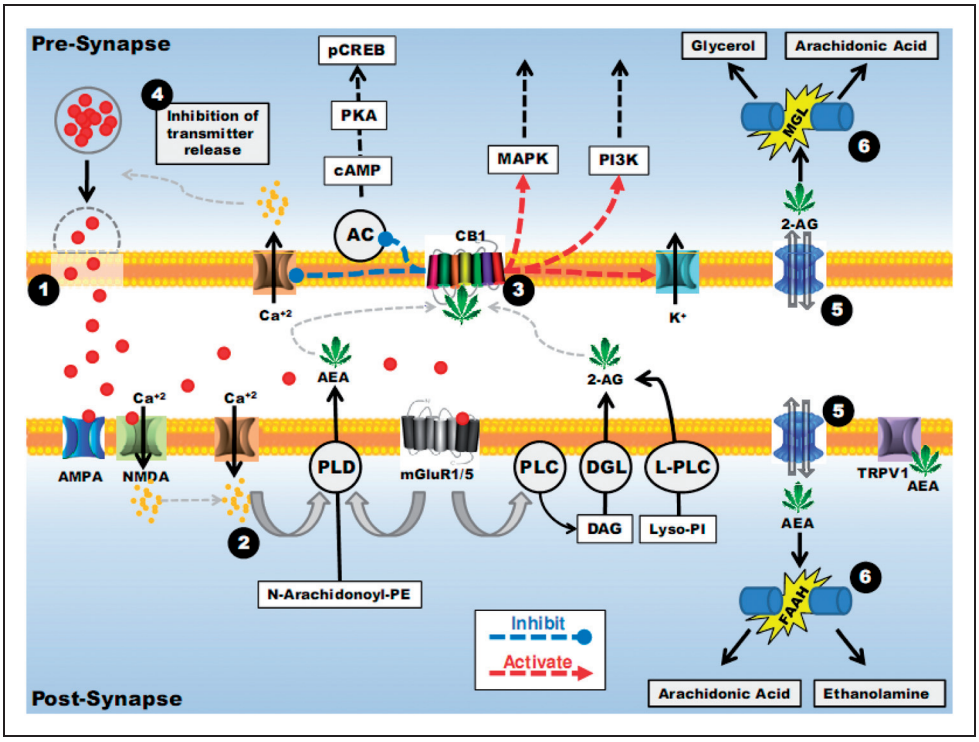

Figure 5. Dynamics of endocannabinoid signaling. This figure is a schematic representation of a glutamatergic synapse. One particular characteristic of the endocannabinoids is that unlike the most common classes of neurotransmitters they are not stored in vesicles. Instead, they are synthesized and released on demand, in a dynamic signaling process that allows action over a very restricted temporal–spatial range. The first evidence for this unusual release mechanism was the demonstration that AEA is not present in resting cells, but it is produced upon stimulation such as neuronal depolarization or following induction of an inflammatory process by bacterial lipopolysaccharides (1). Mobilization of phospholipid precursors from the cell membrane is followed by a Ca^{+2}-dependent enzymatic step that generates either AEA or 2-AG, depending on the stimulation route (2). Notably, besides being an endocannabinoid, 2-AG is also an intermediate in phosphoglyceride metabolism, which may be why this molecule is abundantly found in resting cells. The activation of a postsynaptic cell induces production of endocannabinoids, which travel across the cell membrane and are released in a retrograde fashion by facilitated diffusion in the synaptic cleft. High-affinity binding sites (CB1 receptors) are likely found in the presynaptic terminal of inhibitory or excitatory cells (3) and upon activation further release of glutamate is inhibited (4). Following receptor activation, the endocannabinoid is removed from the synaptic cleft through a selective, saturable, and temperature-dependent system of reuptake (5). Both AEA and 2-AG share a similar reuptake system, therefore suggesting a common cellular transporter, but one that has not yet been completely characterized. Once taken inside the cell, degradation enzymes (FAAH for AEA and MAGL for 2-AG) provide rapid endocannabinoid metabolism (6). There is evidence that within the cell endocannabinoids can also elicit cellular responses that result in further endocannabinoid synthesis, for instance via TRPV1 receptors. AEA, anandamide; 2-AG, 2-arachydonoyl-glycerol; PLD, N-acyl phosphatidylethanolamine phospholipase D; PLC, phospholipase C; DAG, diacylglycerol; DGL, diacylglycerol lipase; L-PLC, liso phospholipase C; L-PI, liso inositol phosphate; AC, adenylyl cyclase; PKA, protein kinase A; pCREB, phosphorylated CREB transcription factor; MAPK, mitogen activated protein kinase; PI3K, inositol triphosphate kinase; FAAH, fatty acid amide hydrolase; MGL, monoacyl glycerol lipase. Black arrows indicate movement, gray dashed arrows indicate the site of action, red dashed arrows indicate activation, blue dashed arrows indicate inhibition. Red small circles represent the excitatory neurotransmitter glutamate. A similar mechanism can be drawn for inhibitory GABAergic synapses.

mechanism that may mediate synaptic fine tuning induced by CB1 receptors activation (Bosier et al., 2010).

The efficacy of CB1 agonists depends on their interaction with G proteins and their intracellular partners, whose expression and recruitment may vary in distinct cell types. Accordingly, the effects of CB1 receptor activation differs depending on the endocannabinoid molecule and the tissue or cell type in which the receptor is expressed (Glass and Northup, 1999). AEA functions as a partial agonist, thereby inducing intermediate G-protein coupling and signal transduction. In contrast, 2-AG is a full agonist that induces a full response in a given tissue preparation (Gonsiorek et al., 2000; Hillard et al., 1999; Savinainen et al., 2001; Sugiura et al., 1995). This might have important consequences for the endogenous modulation of neuronal excitability, depending on which endocannabinoid is released. The capacity of CB1 receptors to mediate quantitatively or qualitatively distinct actions for distinct ligands has been called 'functional selectivity', and this process may be explained by the induction of distinct conformational changes in the receptor's structure or the 'selection' of distinct conformations from a pool of heterogeneous receptors (Howlett, 2005; Mackie, 2008). Ligand-dependent changes in the conformational states of the population of CB1 receptors have been predicted

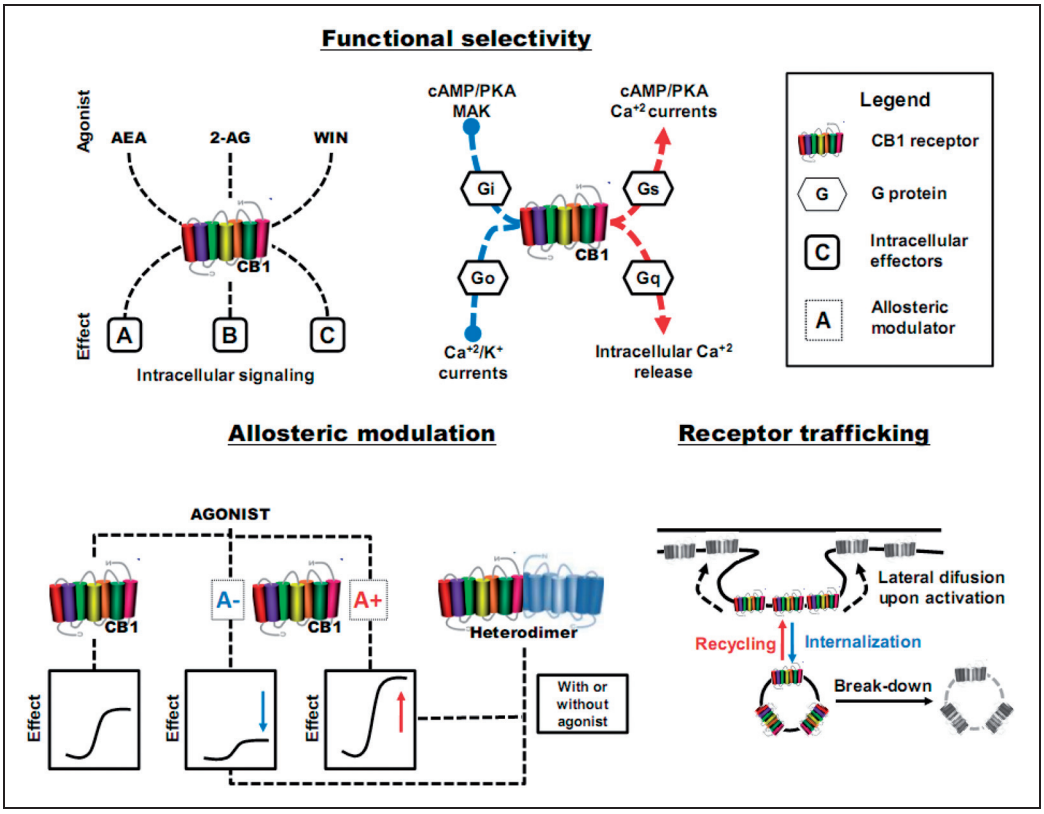

Figure 6. Novel mechanisms of endocannabinoid action. Functional selectivity, allosteric modulation, and receptor trafficking are recently described mechanisms that regulate intracellular signaling by CB1 cannabinoid receptors. Functional selectivity means that different agonists may recruit different secondary mediators, thereby inducing different intracellular effects. Allosteric modulation may occur via site-specific modulation of CB1 signaling by allosteric ligands (bound at a different site than the primary orthosteric site) or via receptor dimerization. Receptor trafficking refers to mechanisms of desensitization involving lateral diffusion, internalization (with possible recycling), and breakdown (leading to down-regulation via receptor degradation). This figure illustrates the mechanisms discussed in the section 'Future directions for endocannabinoid research', pointing to very recent advances in the field. For more details, please read the whole session. Abbreviations are the same as in Figure 5.

by molecular modeling (Shim and Howlett, 2006). The core idea is that different endocannabinoid ligands may activate distinct signaling pathways, depending on their affinity for one of the several possible conformational states of the CB1 receptor. The impact of functional selectivity on the endocannabinoid system has been reviewed in Bosier et al. (2010), wherein the authors explore possible innovative therapeutic opportunities that are a consequence of this process. As such, the emerging picture of CB1-mediated intracellular processes is far more complex than previously believed.

Functional selectivity may, at least in part, be related to the existence of several binding sites on cannabinoid receptors, as has been suggested by structural modeling (Reggio, 2003). An allosteric binding site for the modulation of the CB1 receptors has been described (Price et al., 2005). However, there is not yet any information regarding any putative interactions between endogenous ligands and this site, whether the ligands are endocannabinoids or not. Conceptually, the term allosterism refers to the functional cooperation between different molecules acting at different sites on the same receptor (or oligomeric protein), and the allosteric modulator is the ligand acting at a different site than the orthosteric (principal) receptor binding site (Christopoulos and Kenakin, 2002). The Org27596 and Org29647 compounds were the first CB1 allosteric modulators to be discovered (Price et al., 2005). As allosteric modulators, these compounds have interesting characteristics. They enhance the affinity and reduce the efficacy of ligands that act at the orthosteric site in a non-competitive manner, and they have ligand-dependent effects (Price et al., 2005). Moreover, the effects of these allosteric compounds depends on whether the orthosteric cannabinoid ligand is an agonist or an antagonist. Specifically, they increase the affinity of the agonist [^{3}H]CP55940 but reduce the affinity of the CB1 antagonist [^{3}H]SR141716A (Price et al., 2005). For example, PSNCBAM-1 is an allosteric modulator of CB1 receptors that in a non-competitive manner enhances the affinity but reduces the efficacy of the agonist [^{3}H]CP55940 (Horswill et al., 2007). The *in vivo* effect of PSNCBAM-1 was to reduce food intake nearly as effectively as the CB1 antagonist SR141716A (rimonabant), suggesting a possible therapeutic application (Horswill et al., 2007).

The pharmacological profile of these new drugs suggests the possibility of 'intelligent' antagonists. These drugs would increase the affinity of an agonist, such as an endocannabinoid, for the CB1 orthosteric site while concurrently reducing the capacity of the agonist–receptor complex to induce intracellular responses. Consequently, the agonist functions as an antagonist. As such, an intelligent antagonist could sense variations in the microenvironment surrounding the receptor and adjust its response to these alterations. For example, the allosteric modulator would be more effective at high concentrations of the agonist in the synaptic cleft (Kenakin, 2009). This is certainly a groundbreaking discovery. In addition, the legacy of this research line may not just be the allosteric compounds themselves, but also the recognition and characterization of the allosteric site of CB1 receptors, and its possible influence on the endocannabinoid system. Similar to the discovery of Δ^9-THC followed by AEA, the identification of synthetic allosteric modulators may be just the very first step towards the discovery of endogenous CB1 allosteric modulators.

CB1 receptors may also be allosterically modulated by receptor dimerization. This direct protein–protein interaction involves the formation of transient chemical bonds that may result in functional consequences for each protein. An allosteric interaction occurs when the complex of receptors shows different functionalities to the isolated receptors. The CB1 receptor seems to be a rather promiscuous receptor, forming homodimers with up to four subunits of other CB1 receptors and heterodimers with D2 dopamine receptors, μ opioid receptors, A2A adenosine receptors, β2 adrenergic receptors, and OX1 orexin receptors (Carriba et al., 2007; Ellis et al., 2006; Kearn et al., 2005; Rios et al., 2006; Wager-Miller et al., 2002). These multimeric interactions interfere with various functional aspects of CB1 receptors signaling. Dimerizations between CB1 and D2 receptors can occur even in absence of ligands. However, CB1 agonists tend to increase and inverse agonists tend to decrease dimerization between CB1 and D2 receptors (Kearn et al., 2005). Furthermore, by acting on CB1–D2 dimers, CB1 agonists reverse adenylate cyclase inhibition and MAP phosphorylation induced by D2 agonists, and thereby they produce an overall effect that is opposite to the effect produced by agonism of individual CB1 receptors (Glass and Felder, 1997; Kearn et al., 2005). CB1–D2 dimerization promotes coupling with stimulatory G_s proteins rather than coupling with inhibitory $G_{i/o}$ proteins, which are classically associated with CB1 functions (Jarrahian et al., 2004). A similar phenomenon occurs when CB1 dimerizes with μ opioid receptors (Rios et al., 2006).

In contrast, dimerization between CB1 and β2 adrenergic receptors increases coupling to inhibitory G_i proteins and largely blocks coupling to G_s, in a manner that is independent of ligand binding (Hudson et al., 2009). Similarly, coactivation of CB1 and A2A adenosine receptors enhances coupling with inhibitory G_i proteins and reduces forskolin-induced production of cAMP compared with activation of each receptor alone. In this case, however, CB1–A2A dimerization generates a physical structure that prevents coupling of G_i proteins in the absence of A2A agonists (Carriba et al., 2007). CB1 receptors also form functionally active heterodimers with OX1 orexin receptors (Hilairet et al., 2003). The potency of orexin A (the endogenous agonist of OX1 receptors) is increased approximately 100 fold when OX1 is coexpressed with CB1 receptors in hamster oocytes, suggesting cooperation via receptor dimerization (Hilairet et al., 2003). Indeed, treatment of oocytes coexpressing OX1 and CB1 with the CB1 antagonist SR141716A induces a reduction in the effects of orexin A, whereas treatment with the OX1 antagonist SB-674042 reduces the potency of WIN 55,212-2, suggesting mutual reversibility by the allosteric modulation (Hilairet et al., 2003). Curiously, CB1 receptors also form homodimers with up to four subunits of other CB1 receptors. The functional importance of this form of dimerization remains unknown (Mackie, 2005).

Another aspect of endocannabinoid pharmacology that has been recently studied is the mobility of cannabinoid receptors into and out of the synaptic region. This may occur through endocytosis, desensitization, or receptor internalization. Furthermore, receptor mobility may have important implications for pharmacodynamic tolerance, and it may mediate the plasticity of this receptor system (Martin et al., 2004). Behavioral and physiological tolerance to cannabinoids develops rapidly, and it is primarily mediated by changes in CB1 receptors (Bass and Martin, 2000). Prolonged exposure to a drug acting at metabotropic receptors leads to decreased cellular responses over time, despite the continuing presence of the agonist. This results in the need for a higher amount of drug to generate the same response, a process known as desensitization. Cannabinoid receptors desensitize especially quickly after exposure to high doses or repeated treatments with agonists (Pertwee, 1997). Chronic treatment has generally been associated with a fast decrease in the functionality of CB1 receptors, decreases in CB1 binding sites, or both (Breivogel et al., 1999), suggesting that functional desensitization is intimately linked to receptor internalization (Garland et al., 1996). Therefore, studying receptor desensitization and internalization is critical for the understanding of the implications of chronic use of *Cannabis* or cannabinoid-based medicines (Daigle et al., 2008). Despite its importance, it is still unclear whether tolerance to cannabinoids results from receptor uncoupling, endocytosis, internalization (down-regulation), or a combination of these mechanisms. CB1 receptors undergo constitutive or agonist-induced trafficking between the plasma membrane surface and endosomes, which function as an intracellular receptor reserve. Similar to a number of other metabotropic receptors, CB1 receptors move to caveolae and lipid rafts when they are ready for activation and move out of the rafts after agonist binding, and they subsequently internalize via pathways that depend on clathrin-coated pits (Chini and Parenti, 2004; Sarnataro et al., 2005). This process is reversible after short-term activation, requires the distal carboxy tail of CB1 receptors, keeps the receptors available for recycling into the synaptic zone (after ligand dissociation) without further protein synthesis, and requires both acidification of the endosomal compartment and receptor dephosphorylation (Hsieh et al., 1999). In contrast to activation-induced receptor endocytosis, long-term agonist treatment leads to irreversible internalization and receptor down-regulation, which requires de novo protein synthesis for recycling (Hsieh et al., 1999). The ability of cannabinoid agonists to induce alterations in receptor

cycling may differ. The phytocannabinoid Δ^9-THC has a lower efficacy for induction of endocytosis compared with the synthetic agonist WIN 55,212-2. Nevertheless, chronic treatment with the low-efficacy endocytotic agonist Δ^9-THC produces greater receptor desensitization than the high-efficacy endocytotic agonist WIN 55,212-2 (Wu et al., 2008). Moreover, blockade of receptor endocytosis markedly enhances WIN 55,212-2-induced desensitization of CB1 receptors, suggesting that endocytosis counteracts desensitization by favoring receptor recycling and reactivation (Wu et al., 2008). The endocytotic potency of cannabinoids is negatively correlated with their ability to cause receptor desensitization, such that high-efficacy endocytotic agonist activity may result in a fast decline in receptor function, but delay or preclude the development of long-term cannabinoid tolerance (Wu et al., 2008).

Apart from receptor internalization and down-regulation, an interesting mechanism that parallels CB1 receptors desensitization has recently been discovered. Endocannabinoids must be able to bind to CB1 receptors to be effective, and the release of these molecules is spatially and temporally restricted (i.e. on-demand release). Accordingly, a simple lateral shift in the position of CB1 receptors in the membrane surface may represent an interesting and novel mechanism for modulation of endocannabinoid signaling (Mikasova et al., 2008). As described above, prolonged treatment with cannabinoid agonists triggers CB1 internalization, via a clathrin-coated pit pathway, and G protein uncoupling (Wu et al., 2008). Meanwhile, faster mechanisms reduce CB1 signaling without modifying total receptor binding or coupling (Mato et al., 2004). A large proportion of CB1 receptors are regarded as mobile (nearly 80%) in regular conditions, and they engage in a dynamic 'dance' into and out of the synaptic zone. Agonist-induced desensitization increases the fraction of CB1 receptors in extrasynaptic compartments and strongly reduces their mobility. This process is time dependent, occurs relatively rapidly, and is dependent on CB1 receptor activation (Mikasova et al., 2008). Desensitized CB1 receptors are gradually slowed and immobilized within the extrasynaptic zone, resulting in a progressive loss of synaptic CB1 content. It is not yet known whether extrasynaptic CB1 receptors remain functional, but it is conceivable that their availability for interactions with endocannabinoid molecules is dramatically reduced. Based on this discovery, the concept of agonist-induced desensitization may be modified to comprise not only a reduction in the number of surface CB1 receptors but also a decrease in the synaptic fraction of CB1 receptors and an increase in the fraction of immobile CB1 receptors in the extrasynaptic compartment (Mikasova et al., 2008).

Concluding remarks

The last five decades have seen increased legal constraints on the medical applications of *Cannabis*-derived medicines. Fortunately, these constraints were not sufficient to preclude the advancement of scientific knowledge regarding the phytochemistry and mechanisms of action of *Cannabis*, which has resulted in the discovery of the endocannabinoid neuromodulatory system. Today, we challenge the global community to look at the scientific data and rethink this decision. Not only has *Cannabis* itself been shown to have several potential medical applications, but several other compounds that act on the endocannabinoid system may be shown in the very near future through translational medical research to be effective therapeutics. While the medical use of the *Cannabis* plant has apparently been abandoned in several countries, despite countless reports confirming its safety and the near absence of toxicity, synthetic cannabinoid-based products have curiously been increasingly accepted by the medical community (e.g. the synthetic Δ^9-THC, dronabinol). The disregard of plant products is likely rooted in cultural misconceptions and, controversially, the same psychoactive effects sought by recreational *Cannabis* users. New mechanisms of action (e.g. functional selectivity, allosterism, and receptor trafficking) and evidence-based therapeutic applications of cannabinoids – targeting the wide range of physiological functions affected by the endocannabinoid system – suggests that the careful study of this system will contribute to the development of drugs with higher societal acceptability and likely with fewer side effects.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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