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An Overview on the Biochemistry of the Cannabinoid System

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Abstract About 40 years ago, cannabinoids were considered as the substances responsible for the psychoactive properties of marijuana and other derivatives of *Cannabis sativa*, whereas their medicinal use remained unexplored. However, with the discovery of the endocannabinoid system 20 years later, the compounds able to modify this system are being reconsidered for their therapeutic potential. Thus, the term "cannabinoid" includes now much more compounds than those present in *C. sativa* derivatives, for instance, numerous synthetic cannabinoids obtained by modifications from plant-derived cannabinoids or from the compounds that behave as endogenous ligands for the different cannabinoid receptor types. The term "cannabinoid" should also refer to some prototypes of selective antagonists for these receptors. The explanation for this exponential growth in cannabinoid pharmacology is the discovery and characterization of the endocannabinoid signaling system (receptors, ligands, and inactivation system) which plays a modulatory role mainly in the brain but also in the periphery. The objective of the present review article was to give an overview of the present state-of-the-art of biochemistry of the endocannabinoid system. Other authors in this volume will review their functions in the brain, their alterations in a variety of neurological and psychiatric pathologies, and the proposed therapeutic benefits in these diseases of new cannabinoid-related compounds that improve the pharmacological properties of classic cannabinoids.

Keywords Endogenous · Plant-derived and synthetic cannabinoids · CB_1 and CB_2 receptors · Anandamide · 2-arachidonoylglycerol · Endocannabinoid transporter · FAAH · MAGL

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The Endocannabinoid System. A Brief Account of the Early Steps

The highly lipophilic nature of the components of the plant *Cannabis sativa* prompted the notion that the effects of marijuana were exerted nonspecifically by perturbing membrane lipids [1]. The first important step to challenging that hypothesis was the identification in 1964 by Gaoni and Mechoulam [2] of the correct chemical structure of the psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC), one of the approximately 60 cannabinoids present in the plant [3]. This finding stimulated in the next decade the search for many synthetic analogs, which would be further classified in classic cannabinoids, nonclassic cannabinoids and aminoalkylindols (for review, see [4]). In 1988, Devane et al. [5] demonstrated the existence of specific cannabinoid receptors using the synthetic agonist [^{3}H] CP-55,940 and mouse brain plasma membranes. After the mapping of cannabinoid receptors in the brain by autoradiography [6], Matsuda et al. [7] cloned this G protein-coupled receptor that was identified as the brain receptor for cannabinoids and named CB_1 later on. This receptor was the most abundant receptor in the mammalian brain. It is also present at much lower concentrations in several peripheral tissues and cells. A second cannabinoid receptor, CB_2 [8], is

mainly present in cells of the immune and hematopoietic systems, but it has been recently found also in the brain and other tissues (see [9] for a review). The presence of additional cannabinoid receptors has also been suggested (see later).

Mimicking the process followed in the 70s when it was shown that compounds of the plant *Papaver somniferum* (opium) activated certain membrane receptors with no endogenous known ligand, which led to the discovery of enkephalins, β-endorphins and dynorphins [10], during the 90th decade, there was a search for endogenous ligands for cannabinoid receptors. In 1992, Mechoulam et al. [11] isolated and characterized the lipid arachidonoylethanolamide (anandamide), the first endogenous compound found to bind brain cannabinoid receptor with relatively high affinity. In 1995, another arachidonic acid derivative, 2-arachidonoylglycerol (2-AG) was isolated independently for Mechoulam et al. [12] and Sugiura et al. [13]. Later on, more lipids with endocannabinoids properties have been characterized, but the bulk of studies concerning synthesis, cellular traffic, inactivation, and biological function have focused so far on anandamide and 2-AG (see [14] for an updated historical account).

Presently, the endocannabinoid system represents a therapeutically promising mechanism of intercellular communication, although only partially deciphered [15]. So far, three kinds of basic components have been described: (1) endogenous ligands, (2) membrane receptors, and (3) proteins involved in the inactivation of the endocannabinoid signal, those which remove the ligands through re-uptake and subsequent hydrolysis. Describing the basic biochemistry of the endocannabinoid system components will be the objective of this article.

Endocannabinoid System Ligands

At present, the two endocannabinoid ligands best characterized are anandamide, by far the most studied [16], and 2-AG [17]. Other endocannabinoids are noladin ether (2-arachidonyl glyceryl ether) [18], arachidonoyl dopamine (NADA) [19], and virodhamine [20], but their biological significance and biochemical characteristics are largely unknown.

Anandamide can act as an endogenous ligand for CB_1, CB_2, and vanilloid (TRPV1) receptors. Nevertheless, depending on the tissue and biological response measured, it behaves as a partial or full agonist of CB_1 receptors. It has very low efficiency as CB_2 agonist and may even act as antagonist depending on the G proteins interacting [21]. 2-AG acts as agonist at both CB_1 and CB_2 receptors. As it is active at CB_2 receptors, while anandamide binds poorly to these receptors, some authors considered 2-AG as the true ligand for CB_2 receptors [17]. Its basal levels in the brain are much higher (about two orders of magnitude) than those of anandamide, which suggest that only a fraction of the total is involved in endocannabinoid signaling [13, 17]. Noladin ether shows more affinity for CB_1 receptors than for CB_2 receptors [22]. Virodhamine acts as an antagonist of CB_1 receptors and a partial agonist toward the CB_2 receptor [23], but some authors are critical of its biological relevance [24]. Furthermore, NADA binds to CB_1 receptors with more affinity than to CB_2, and it is also agonist at $TRPV_1$ receptors, which led several authors to consider this compound as an important endovanilloid ligand [25].

In addition to the endocannabinoids, other endogenous fatty acid derivatives have been called "endocannabinoid-like" because they do not activate CB receptors such as anandamide or 2-AG do, but they can enhance the action of classic endocannabinoids at their receptors through a mechanism called "entourage effect" [26, 27]. Among them are palmitoylethanolamide, stearoylethanolamide, oleoylethanolamide, arachidonoylglycine, 2-lineoylglycerol, and 2-palmitoylglycerol.

Plant and Synthetic Ligands of the Cannabinoid System

As the pharmacology of the cannabinoid system is addressed elsewhere in this volume, we will mention exogenous ligands very briefly.

Phytocannabinoids

Among the more than 60 cannabinoids of the plant *C. sativa*, the first isolated and best known is Δ^9-THC [2], the psychoactive component. It is agonist at both CB receptors and it partially mimics the actions of the endogenous cannabinoids [4]. Cannabidiol (CBD) is not a ligand for any of the two CB receptors, but it shows important cannabimimetic actions attributed to antioxidant properties, inhibition of anandamide degradation, and/or interactions with other cannabinoid receptors still unidentified [3]. The therapeutical potential of the rest of the components remains nearly unexplored [28, 29]. Moreover, the finding that species different to *C. Sativa* have components able to bind to CB receptors open new and interesting possibilities for research [30].

Synthetic Ligands

The synthetic cannabinoid receptor ligands are characterized by a wide chemical diversity. They are usually classified into the following categories: classical cannabinoids, nonclassical cannabinoids, aminoalkylindoles, and eicosanoids [4]. It should be noted that several agonist (JTE 907, BAY 38-7271) and several antagonists (diarylpyrazoles as SR141716A) do not fall into these standard classes [4].

The classic cannabinoid agonists retain the tricyclic diterpene structure of Δ^9-THC. This category includes compounds like HU-210, HU-234, nabilone, (−)-5′-(1,1′-dimethylheptyl) cannabidiol (DMH-CBD) [9, 31] and ajulemic acid [32, 33], which act at both CB receptors. To avoid side effects to the central nervous system, selective agonist for CB_2 receptors, which are located mainly in the periphery, have been developed [34]. These agonists include compounds like JWH-133, JWH-1051, JWH-308, L-759633, and L-759656. These compounds could be useful in the treatment of pain and inflammation. Nevertheless, CB_2 receptors are also expressed in the microglia during inflammatory states [35] and in neural progenitors since late embryonic stages [36], which would broaden the therapeutical applications of their selective ligands.

In nonclassic cannabinoids agonists, one of the rings of the tricyclic Δ^9-THC structure is open. Among them are CP55940, CP47497, CP55244, and the CB_2 selective agonist HU-308 [4, 34, 37, 38].

The aminoalkylindoles are less related to phytocannabinoids. This category includes compounds like WIN 55,212-2 [39] and the selective CB_2 agonists JWH-015, L-768242 and AM 1241 [40].

There are a number of synthetic eicosanoids, mainly derived from anandamide structure, that improve several pharmacokinetic (they are metabolically more stable than anandamide) or pharmacodynamic (they provide more selectivity for the different CB receptors or are hybrid CB/TRPV1) [4, 34, 41, 42, 43]. Examples of these analogs are R-methanandamide (AM356), arachidonoyl-2-chloroethylamide (ACEA), arachidonoyl-ciclopropylamide (ACPA), O-1812, retroanandamide, arvanil, O-1861, O-585, and O-689.

In relation with antagonists [4, 34] SR141716A (rimonabant) was the first CB_1 antagonist to be developed and is going to be approved in Europe for the treatment of obesity. It behaves as CB_1 inverse agonist. Other inverse agonists are SLV-326, LY320135, and AM251. Examples of neutral antagonists are O-2654 and AM5171. As regards to CB_2 receptors, AM630, SR144528, and O-1184 are the most characteristic antagonists. Therapeutic areas for cannabinoid antagonists include obesity, drug addiction, and perhaps central nervous system (CNS) disorders. Furthermore, inhibitors of biosynthetic enzymes might show parallel characteristic to cannabinoid receptor antagonists as the recent inhibitors for 2-AG biosynthesis described by Bisogno et al. [44].

Other interesting CB-related compounds are those capable of blocking specific elements of the mechanism of termination of biological signal of the cannabinoid system. These are called indirect agonists and offer the possibility of obtaining new drugs devoid of the side effects associated with direct CB_1 agonists. The indirect agonists developed so far are mainly re-uptake blockers [45, 46, 47] and FAAH inhibitors [9, 14]. Indirect agonists seem promising to treat pain, some motor impairments, cancer cell proliferation, anxiety, and hypertension, among others [14].

Biosynthesis of Endocannabinoids

Like eicosanoids, endocannabinoids are formed and released locally on demand. Therefore, their levels are not constant, and the kinetic of their degradation are a major factor in their activity. The in vivo synthesis of anandamide is believed to occur through the enzymatic hydrolysis by phospholipase D of the membrane precursor *N*-arachidonoyl-phosphatidylethanolamine (NAPE) [48]. NAPE is formed by the enzymatic transfer of arachidonic acid in the *sn*-1 position of phosphatidylcholine to the amine group of phos-

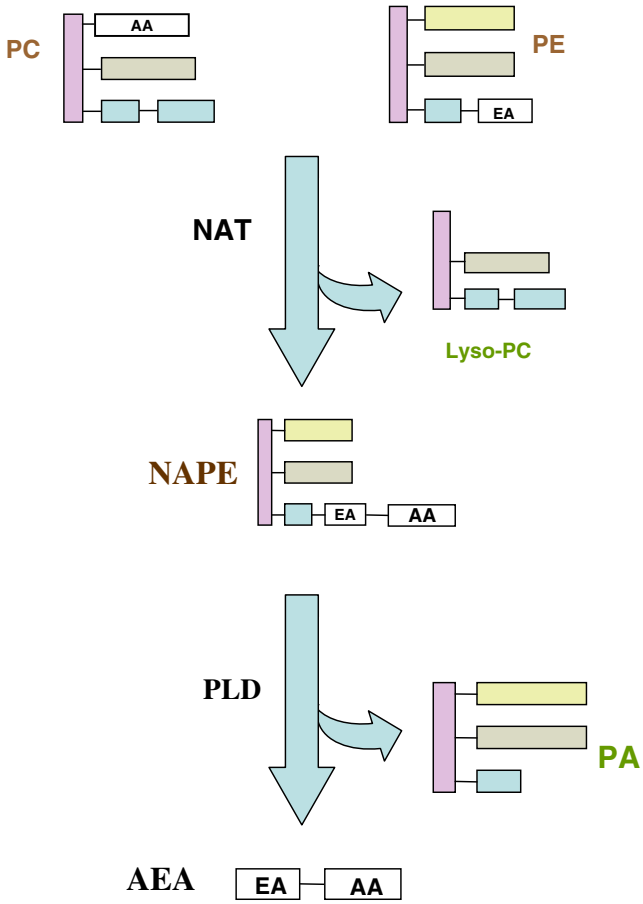

Fig. 1 Major pathway for the biosynthesis of anandamide. The first step is catalyzed by *N*-acyltransferase. This enzyme transfers the arachidonic acid from phosphatidylcholine to phosphatidylethanolamine, forming *N*-arachidonoyl-phosphatidylethanolamine. In the next step, the phospholipase D cleaves NArPE in anandamide and phosphatidic acid. *PC* Phosphatidylcholine, *PE* phosphatidylethanolamine, *NAT* *N*-acyltransferase, *EA* ethanolamine, *AA* arachidonic acid, *NArPE* *N*-arachidonoyl-phosphatidylethanolamine, *PLD* phospholipase D, *AEA* anandamide, *PA* phosphatidic acid

phatidylethanolamine (Fig. 1) [49, 50]. No specific transacylase has yet been identified, but a specific NAPE-PLD has been cloned [51]. Other variants to this pathway have been suggested (see [9] for review). In the stomach, a secretory phospholipase A_2 can catalyze the conversion of *N*-acylphosphatidylethanolamide to *N*-acyl-lyso phosphatidylethanolamide. The latter is metabolized by a lyso-PLD, and several *N*-acylethanolamides are formed, including anandamide [52]. Another alternative pathway has been described in RAW246.7 macrophages involving hydrolysis of NAPE to phosphoanandamide by a PLC and subsequent action of a phosphatase [53].

2-AG is synthesized from diacylglycerol (DAG) by DAG lipase selective for the *sn*-1 position (Fig. 2). DAG can be generated either from phosphoinositides by a specific PLC or from phosphatidic acid (PA) by PA phosphohydrolase. The two isozymes of DAG lipase (α and β) have been cloned [54]. The two forms of DAG lipase are located in the postsynaptic plasma membrane in the adult brain.

Endocannabinoids are not stored in synaptic vesicles like others neurotransmitters. Instead, they are immediately released by "postsynaptic elements" to the synaptic cleft where these compounds are able to bind to several membrane receptors (Fig. 3) frequently located presynaptically. Concordant with this location of cannabinoid receptors is the proposal that endocannabinoids may act as retrograde signal molecules at synapses (see [55] for review). Therefore, it has been described that the signaling induced by binding of anandamide or 2-AG to cannabinoid receptor controls presynaptic events as the release of several neurotransmitters, mainly GABA and glutamate [56], but also extends to other neurotransmitters [57, 58].

Cannabinoid Receptors

Two cannabinoid/endocannabinoid receptors have been cloned and characterized [5, 7, 8]. However, there is evidence about the existence of other potential cannabinoid receptor types [59]. This complexity has been related to several factors: (1) the lack of understanding of the regulation of the cannabinoid receptor genes and the molecular identity of the other types and (2) the occurrence of splicing variants. The two better characterized endocannabinoid receptors have been called: (1) CB_1 (present ubiquitously and preferentially in the brain and spinal cord, although also present in the periphery) [5, 6] and (2) CB_2 (located peripherally, almost exclusively in the immune system, although recently also identified in the normal and pathological brain) [61, 62, 63, 64, 65]. CB_1 receptor appears highly conserved across species [66], whereas the CB_2 receptor shows more cross-species variation. They belong to the superfamily of seven transmembrane-domain

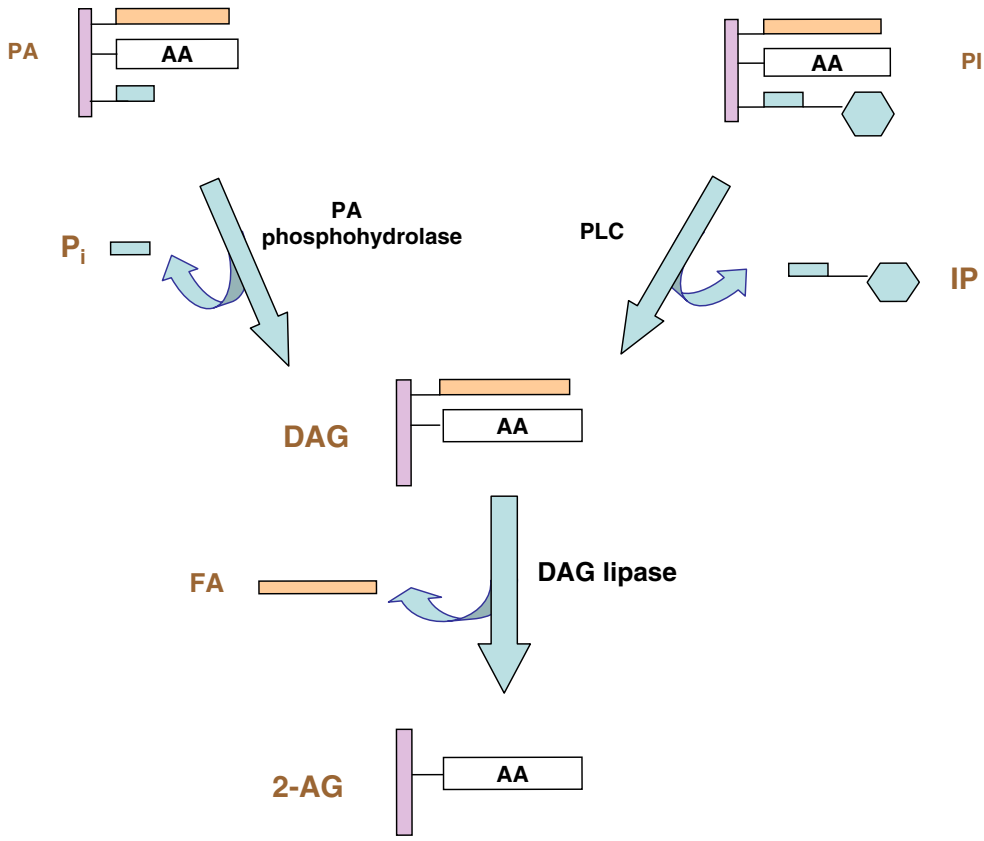

Fig. 2 Major pathways for the biosynthesis of 2-AG. The DAG, the main precursor of 2-AG, can be synthesized by two pathways. In the first, the phosphatidic acid is hydrolyzed by a specific phosphatase to give DAG. The other pathway requires the hydrolysis of the precursor, phosphatidylinositol, catalyzed by the enzyme phospholipase D. In both cases, the sn-1 selective DAG lipase catalyzes DAG hydrolysis to 2-AG. *PI* Phosphatidylinositol, *AA* arachidonic acid, *IP* inositol phosphate, *PLC* phospholipase C, *DAG* diacylglycerol, *PA* phosphatidic acid, *Pi* inorganic phosphate, *FA* fatty acid, *2-AG* 2-arachidonoylglycerol

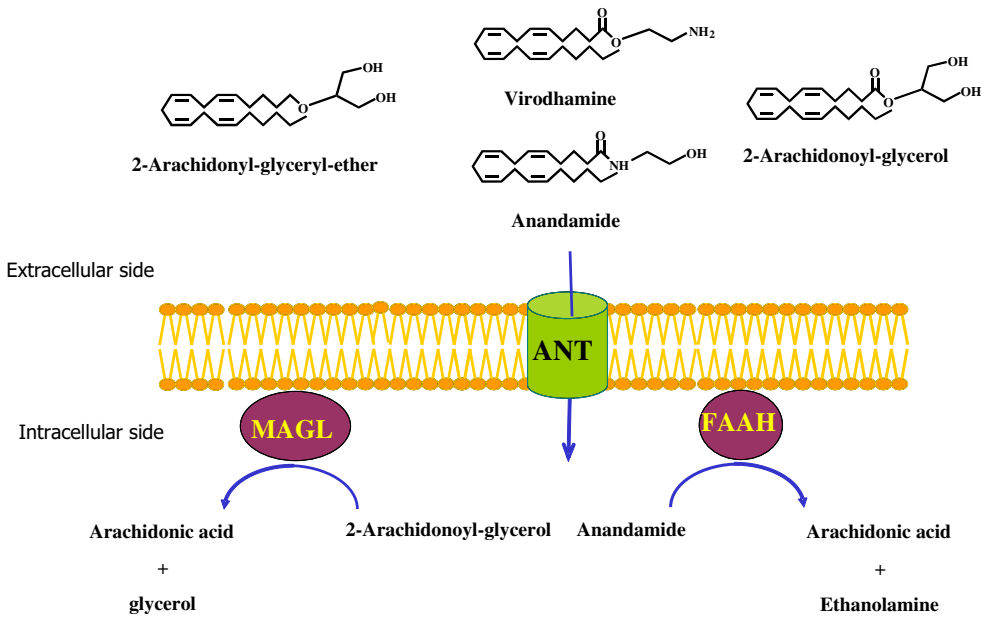

Fig. 3 Mechanisms proposed for the endocannabinoid inactivation. Once re-uptaken by cells, AEA and 2-AG are hydrolyzed by intracellular enzymes to arachidonic acid and ethanolamine or glycerol respectively. *ANT* Anandamide transporter, *MAGL* monoacylgycerol lipase, *FAAH* fatty acid amide hydrolase

GTP-binding protein-coupled receptors (Fig. 3). On the other hand, although some studies have suggested that endocannabinoid ligands might have certain selectivity for the different cannabinoid receptor types, in general, all ligands are able to bind and activate both receptor types in vitro, although they exhibit a certain selectivity related to the sites where these endocannabinoids are produced [60].

As mentioned above, other types of CB receptors, not yet completely characterized, have also been suggested. A special mention should be done for the receptor "orphan G-protein-coupled receptor GPR55" (G protein receptor, GPR) [67]. This receptor shows different expression between species. GPR55 presents a variant termed GPR55A, which does not activate Gi/o or Gs proteins. These data suggest that this might be a new type of cannabinoid receptor with ligand binding and signalling profiles distinct from those for CB_1 and CB_2.

An other interesting alternative receptor has been referred to as "abnormal cannabidiol receptor" whose major ligand is the abnormal cannabidiol (abn-cbd) [68]. Abn-cbd is a neurobehaviorally inactive cannabinoid, which does not bind to CB_1 receptor but causes effects in wild-type mice and in CB_1 knockout and CB_1/CB_2 knockout mice. In addition, its effects were not due to activation of vanilloid TRPV1 receptor (see next paragraph).

Interaction Between the Cannabinoid and Vanilloid Systems

The vanilloid VR1 receptor was initially described as molecular integrator of nociceptive stimuli abundant in sensory neurons (for review, see [69]), but it has been recently located in many brain structures [70]. This receptor belongs to the family of ion channel receptor and is activated by capsaicin and resiniferatoxin [71]. Anandamide, as well as other related compounds, such as AM404, the inhibitor of the anandamide transporter, may also bind TRPV1 receptors, thus, representing an alternative target for this endocannabinoid in the control of specific brain functions [72]. Therefore, CB_1 and TRPV1 receptors are coexpressed on a subpoblation of primary sensory neurons whose activation by capsaicin induces anandamide production and release [73, 74].

All of these data point to a cross-regulation between both systems. Even for some authors, vanilloid receptors could be the ionotropic receptor type for the endocannabinoid system, whereas the CB_1 and CB_2 receptor types may serve as metabotropic receptor inside the "cannabinoid receptor family" [75].

Intracellular Signalling Coupled to the Activation of CB Receptors

Cannabinoid receptor activation of G-proteins influences multiple effector systems. CB_1 receptors are a member of the superfamily of seven transmembrane receptors that are coupled to Gi/o proteins and, under specific conditions, also to Gs. By coupling to Gi/o proteins, CB_1 receptors regulate the activity of Ca^{2+} and K^+ channels, inhibit adenylyl cyclase, and activate the ERK-MAP kinase pathway. CB_2 receptors also couple to Gi/o proteins and regulate the activity of several signal transduction pathways that operate through the adenylyl cyclase/cAMP system and the ERK-MAP kinase pathway in the same way that CB_1 receptor does (for review, see [59, 76]).

Signal Transduction Mechanisms of CB_1 Receptors

In general, cannabinoids inhibit adenylyl cyclase via Gi proteins. This has been demonstrated in several cell types and in brain membranes (for review, see [77]). However, in certain circumstances, CB_1 can be coupled to Gs instead of Gi and elicit cAMP accumulation [78, 79]. One possible explanation for this duality is the demonstration of the existence of multiple conformations of the CB_1 receptor, induced for different agonists, with variable affinities for different G proteins [80, 81]. The isoforms of adenylyl cyclase present in the cells examined can also be an important contribution to the dual effect of cannabinoids on cAMP. Therefore, it has been reported that coupling of CB_1 to adenylyl ciclase isoforms 1,3,5,6, or 8 resulted in inhibition of cAMP through the release of Giα subunits, whereas coupling of CB_1 to adenylyl ciclase isoforms 2,4, and 7 produced an increase of cAMP through the release of G$\beta\gamma$ subunits [79].

Cannabinoids decrease Ca^{2+} conductance (primarily N- and P/Q-type Ca^{2+} channels) and increase K^+ conductance (voltage-dependent ion channels, Kir and A-type K^+ channels) [82, 83]. This regulation of ion channels is thought to underlie the cannabinoid-induced inhibition of neurotransmitter release at presynaptic sites. Most of these effects are mediated through the CB_1 receptor, although there is evidence to suggest that cannabinoid can modulate ion channel function directly by a receptor-independent-mediated mechanism [84]. As regards to the effects of endocannabinoids on intracellular calcium, early studies reported non-receptor-mediated effects, whereas more recent studies have demonstrated CB_1-receptor-mediated activation of intracellular calcium stores [85]. The key determinant between the production of receptor and non-receptor effects appears to be the type of cells used.

The increase in intracellular Ca^{2+} in the presence of decreased conductance to this ion is probably due to release of Ca^{2+} from intracellular sites. Thus, CB_1 receptor stimulation also appears coupled to PLC activation, through Gi/o proteins, leading to increased levels of inositol 1,4,5-trisphosphate for the induction of Ca^{2+} release from internal stores [86]. Other possible way to increase intracellular Ca^{2+} via PLC is through coupling to $Gq/_{11}$ proteins [85].

Probably related to the intracellular Ca^{2+} increase elicited by endocannabinoids is the observation that activation of CB_1 receptors stimulates constitutive nitric oxide synthase whose isoforms are Ca^{2+}-calmodulin-dependent. In line with the dual effects of cannabinoids, stimulation of CB_1 receptors also appear to mediate inhibition of inducible nitric oxide synthase [87, 88].

In addition to the above signaling mechanisms, CB_1 receptors have been also shown to link positively to MAP kinase [89, 90]. The MAP kinase pathway is a key signaling mechanism that regulates many cellular functions such as cell growth, transformation, and apoptosis. Its activation is normally associated with the initial activation of a tyrosine kinase-linked receptor. This activates the intracellular G protein Ras and sets up signaling cascades leading to the phosphorylation and activation of MAP kinase (also named ERK, extracellular signal-regulated kinase), which can phosphorylate various cytoplasmic nuclear proteins. Furthermore, MAP kinase activation can be linked to expression of immediate early genes.

There are two signal transduction pathway proposed for the activation of MAP kinase by the CB_1 receptor. The first involves the activation of PI3K/PKB, which in turn mediates tyrosine phosphorylation and activation of Raf [91]. The second pathway is initiated by sphingomyelin hydrolysis, release of the lipid second messenger ceramide, and the subsequent activation of the Raf MAP kinase cascade [92].

The stimulation of CB_1 receptors may also regulate MAP kinase activity indirectly through its effects on cAMP accumulation. A decrease in cAMP levels and consequently in PKA activity, may participate in the stimulatory effects of CB_1 receptor activation on the MAP kinase pathway. CB_1 receptor inhibition of cAMP mediates ERK and focal adhesion kinase (FAK) activation, with Fyn as a common link between them [91].

Signal Transduction Mechanisms of CB_2 Receptors

CB_2 receptor inhibits adenylyl cyclase activity through Gi/o proteins. However, there is no evidence that this receptor is also coupled to Gs [93].

CB_2 receptors also produce the activation of p42/p44 MAP kinase (ERK). The pathway proposed is by activation of PI3K/PKB, which in turn induces translocation of Raf-1 to the membrane and phosphorylation of p42/p44 MAP kinase [94]. There is also evidence that the CB_2 receptor induces the expression of genes through a PKC-dependent activation of MAP kinase [95].

As regards to the modulation of intracellular Ca^{2+}, the stimulation of CB_2 receptors initiates a rise in Ca^{2+}. It is important to note that this effect is not mediated by changes in voltage-dependent or ligand-dependent Ca^{2+} channels, but it results from the activation of PLC and a subsequent release of Ca^{2+} from IP_3-sensitive stores [96] (Fig. 4).

Mechanisms of Endocannabinoid Inactivation

To terminate the endocannabinoid signaling, these endogenous compounds, as other neurotransmitters, must be rendered inactivated. This inactivation appears to be regulated by a two-step process: (a) endocannabinoids must be transported

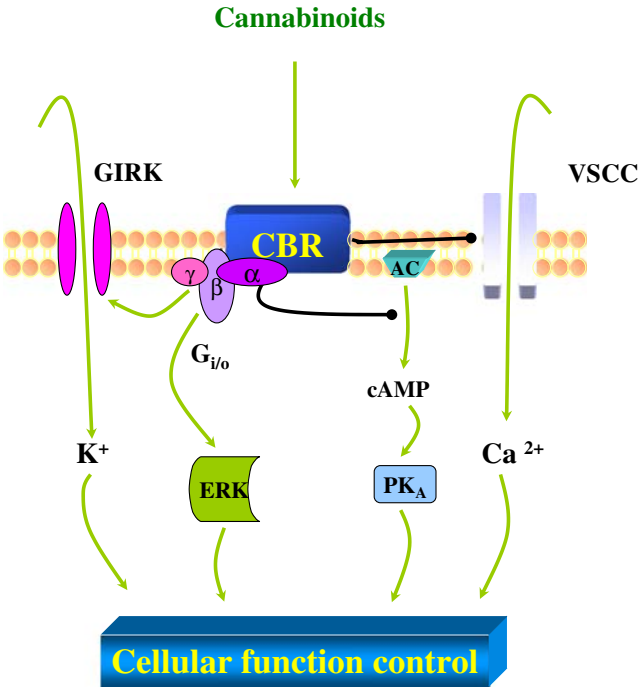

Fig. 4 Major signal transduction mechanisms coupled to the activation of cannabinoid receptors. Agonist-stimulated CB_1 receptors interact with G-proteins to activate them, which permits the dissociation of the α monomer and βγ dimer and activation of specific effectors. *Green lines* indicate activation, whereas *black lines* show inhibition. *CBR* Cannabinoid receptor, *GIRK* G-protein-coupled inwardly rectifying K^+, *MAPK* mitogen-activated protein kinase cascade, *AC* adenylate cyclase, *cAMP* cyclic AMP, *PKA* protein kinase A, *VSCC* voltage-sensitive Ca(2+) channel

into cells by a facilitated transport system [97] and (b) then hydrolyzed by the action of several enzymes [98]. Anandamide is hydrolyzed to its two components (arachidonic acid and ethanolamine) by the action of an amide hydrolase selective for fatty acid amides, called "fatty acid amide hydrolase" (FAAH) [99, 100], whereas 2-AG is degraded to arachidonic acid and glycerol by the action of a monacylglycerol-lipase (MAGL) [101, 102]. The mechanisms of inactivation of the rest of endocannabinoids are less well known.

Endocannabinoid Uptake

The endocannabinoid uptake is critical for understanding the mechanism of inactivation of these compounds. The first study demonstrating that anandamide is taken up into the cells and metabolized was reported by Deutch and Chin [103] in 1993. Di Marzo et al. [104] in 1994 proposed that the uptake of anandamide is a facilitated transport process. Anandamide transport meets four criteria of a carrier-mediated process: saturability, fast rate, temperature dependence, and substrate selectivity [97, 105, 106, 107]. It has also been shown to be independent of ion gradients and ATP hydrolysis [97, 105], but can be affected by nitric oxide [106]. Anandamide uptake has been observed in neurons, glial cells, and multiple cell lines from both CNS-derived and non-neural cells (see [108, 109] for review). Although during recent years there have been considerable efforts to identify the putative endocannabinoid transporter, the protein responsible for this function has not been isolated and/or cloned yet. This has generated certain controversy about the existence of this transporter [110, 111]. To date, it is generally accepted that the movement of anandamide through the cell membrane is a saturable process, and different mechanisms for the uptake of anandamide have been proposed, which include: (1) facilitated diffusion mediated by a membrane carrier [112, 113], (2) simple diffusion driven by FAAH-mediated cleavage of anandamide [110], (3) simple diffusion driven by intracellular sequestration of anandamide [114], and (4) an endocytic process that targets anandamide to intracellular compartments containing FAAH [115].

In contrast to the wealth of information concerning the cellular uptake of anandamide, there is little available data published about the 2-AG uptake. Few studies have proposed that 2-AG enters the cell by specific 2-AG transporter [116, 117] via the putative anandamide transporter or by simple diffusion [118].

Hydrolysis of Anandamide

Once inside the cell, anandamide is hydrolyzed to arachidonic acid and ethanolamine. The hydrolysis of anandamide was first described by Deutch and Chin [103] in 1993. This hydrolysis is mostly attributed to a membrane-bound amidase generally referred to as "fatty acid amide hydrolase", although it is also termed as "anandamide amidase", "anandamide amidohydrolase", or "anandamide hydrolase" [99, 100, 119, 120]. FAAH is an integral membrane bound protein, and its activity is associated with microsomal and mitochondrial membranes [121, 122].

A notable property of FAAH is a wide substrate specificity for long-chain fatty acid derivatives. Although anandamide is the most active substrate, FAAH hydrolyzes the amide-bound other *N*-acylethanolamines of long-chain fatty acids [121], so it lacks the necessary specificity for anandamide.

FAAH is widely distributed in various rat tissues. The specific activity for this enzyme was higher in this order: liver, small intestine, testis, brain, stomach, and kidney [99, 103, 123]. However, organ distribution of human FAAH was markedly different. It was more abundant in the pancreas, brain, kidney, and skeletal muscle [124]. Regional distribution of FAAH has been intensively investigated in rat brain [125, 126, 127]. The hydrolyzing activity of anandamide was high in regions which are also rich in CB_1

receptors as hippocampus, cerebral cortex, and cerebellum, but FAAH did not co-localize with CB_1 in the same types of cells.

The physiological importance of FAAH was evidenced with the recently developed FAAH knockout mice [128]. In these animals, the levels of anandamide and others *N*-acylethanolamines were elevated more than tenfold compared to wild-type animals, and they also displayed higher sensitivity to the biological actions of this endocannabinoid.

Hydrolysis of 2-AG

Although it has been shown that FAAH can catalyze the degradation of 2-AG under some circumstances [129], the main enzyme responsible for the hydrolysis of this compound is a MAGL. The action of this enzyme for the hydrolysis of 2-AG to arachidonic acid and glycerol thus represents the main degradation pathway for this compound, as it has been demonstrated in different mammalian tissues and cells [118, 130, 131]. This enzyme is present in various regions of the brain in which the CB_1 receptor is abundant (hippocampus, cerebral cortex, anterior thalamus, and cerebellum) [101, 102]. Interestingly, MAGL is expressed presynaptically in the adult brain, and the enzyme responsible for the biosynthesis of 2-AG (DAGLs) has been localized postsynaptically. This suggests a major role for this enzyme in the degradation of 2-AG as retrograde messenger [54, 102].

Other Mechanisms of Endocannabinoid Inactivation

In addition to being hydrolyzed by CB-related system enzymes, anandamide and 2-AG have been shown to be metabolized by other types of enzymes involved in the general metabolism of eicosanoids. This is the case of various types of oxygenases that act on arachidonic acid. These include the cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 oxidases that are known to be involved in eicosanoid production from arachidonic acid [132].

Anandamide and 2-AG can be oxygenated by COX-2 to yield prostaglandin-ethanolamine and prostaglandin-glycerol-ester [133, 134]. However, anandamide was found to be a poor substrate for this oxygenase, in contrast to 2-AG which has been shown to be the preferred substrate for COX-2.

12-LOX and 15-LOX have been reported to oxygenate both anandamide and 2-AG to yield 12- and 15-hydroperoxy derivatives of these compounds. However, 5-LOX was almost inactive [132, 135].

The physiological significance of the enzymatic oxygenation of endocannabinoids is not yet clarified [118]. It may represent a simple inactivation pathway for these compounds, as their enzymatic hydrolysis. In contrast, it has been shown that certain oxygenated products are more stable than the parent endocannabinoid and also can bind to the cannabinoid receptors [135, 136]. Thus, oxidative metabolism would represent an activation pathway. The diversity of products formed by anandamide and 2-AG oxygenation has also suggested the possibility that the generated lipids possess potent biological activities distinct from the parent endocannabinoids; so, endocannabinoid oxidation may represent a source of novel signal mediators.

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