The molecular machinery required to process endocannabinoids lipid signaling and their respective receptors

A maquinaria molecular necessária para processar mensageiros lipídicos endocanabinoides e seus respectivos receptores

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ABSTRACT

BACKGROUND AND OBJECTIVES: Pharmaceutical preparations of cannabis have been used by mankind since long time ago, and recently they have been the pharmaceutical industry's focus. However, for proper therapeutic application, in-depth knowledge of the endocannabinoid system, which is made mainly by lipid signaling, is needed. The purpose of this study was to explore the current understanding of the players in this system, paying special attention to the molecular machinery required to process it.

CONTENTS: This is a narrative review of the current literature regarding major components of the endocannabinoid system, in particular: the receptors, main endogenous ligands, and the enzymes responsible for its components processing. The pharmacological and preclinical aspects were emphasized.

CONCLUSION: The better comprehension of the molecular structure of receptors and enzymes will be crucial to developing new pharmacological strategies. A detailed description of the machinery responsible for endocannabinoid lipid metabolization will pave the way for the discovery of new drugs that act on the endogenous system and that can be applied effectively in clinical practice.

Keywords: Cannabinoids, Membrane lipids, Pharmacology.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Os preparados medicinais canabinoides são há muito utilizados pela humanidade e têm sido objeto de interesse da indústria farmacológica recente. Para a aplicação terapêutica adequada é necessário, no entanto, o conhecimento aprofundado do sistema canabinoide endógeno, o qual em sua grande parte é constituído por mensageiros lipídicos. O objetivo deste estudo foi explorar o conhecimento vigente a respeito dos constituintes desse sistema, com especial atenção à maquinaria molecular necessária para processá-los.

CONTEÚDO: Trata-se de uma revisão narrativa da literatura atual acerca dos integrantes do sistema canabinoide endógeno, notadamente: seus receptores, os principais ligantes endógenos e as enzimas responsáveis pelo processamento de seus componentes. Os aspectos farmacológicos e pré-clínicos foram enfatizados.

CONCLUSÃO: O melhor entendimento da ultraestrutura de receptores e enzimas contribuirá de forma decisiva para o desenvolvimento de novas estratégias farmacológicas. A partir da descrição pormenorizada da maquinaria responsável pela metabolização lipídica endocanabinoide é que se pavimentará o caminho para a descoberta de novos fármacos que atuem no sistema endógeno e que possam ser aplicados de forma eficaz na prática clínica.

Descritores: Canabinoides, Farmacologia, Lipídeos de membrana.

INTRODUCTION

Medicinal preparations from the plant *Cannabis sativa* have been used throughout human history[1] as already mentioned in this special issue. However, only recently the psychoactive substance, Δ9-tetrahydrocannabinol (Δ9-THC), was discovered and isolated from hundreds of phytocannabinoids present in the plant[2,3]. This fundamental discovery led to the synthesis of several cannabinoids, which enabled the accumulation of pharmacological knowledge until, two decades after the discovery of THC, the first cannabinoid membrane receptor was identified and cloned, receiving the acronym CB1[4], followed quickly by the discovery of the second cannabinoid receptor CB2[5].

After the discovery of the receptors, it was possible to verify their first endogenous agonists. In 1992, the substance N-araquidonylethanolamine (AEA or anandamide)[6] was recognized. Subsequently, with the fact that AEA cannot completely reproduce the effects verifiable with THC, the second most important endocannabinoid (EC), 2-araquidonylglycerol (2-AG)[7,8], was ar-

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HIGHLIGHTS

- Emphasize the complexity of the endocannabinoid system and go beyond understanding direct pharmacological action.
- Comment regarding the interactions between the endocannabinoid system and other receptor families such as TRPs and PPARs.
- Point out potential promising targets for future research.

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rived at. Both derivatives of arachidonic acid (AA), were the first endogenous cannabinoid substances identified and remain the best studied. Some peptides and derivatives of AA metabolism that generate a cannabinoid-like effect have been recently described and are the target of intense research[9,10].

Thus, synthetically, there is a system formed by two membrane receptors (CB1 and CB2) and two families of lipid signalers that act as their ligands, which, together with the enzymes that synthesize and metabolize them, form the so-called endogenous cannabinoid system (ECs)[11]. This system has some characteristics that allow it to be distinguished from other classical neurotransmitter systems, especially in regard to nociception. Among them, a fundamentally important characteristic is the fact that the machinery related to the processing of lipid EC messengers is located in the synaptic terminals of the nociceptive pathway. Moreover, since ECs are not stored in synaptic vesicles, but produced on demand after intense neuronal activation, the probable ECs role is to brake neuronal signaling in response to its high activation[11]. In this review article, the intent was to explore this machinery components, detailing its constituents and elucidating its main aspects, with special focus on the relationship between ECs and their receptors.

RECEPTORS

CB1 and CB2 receptors belong to the large family of G-protein-coupled receptors (GPCR). It is an extensive and diverse family of membrane receptors responsible for translating external signals (such as light, lipidic and proteinic particles, among others) into specific cellular responses[13]. Currently, the central contributions of these receptors in cell signaling have turned them into a key piece in drug discovery research[12,13]. They are composed of seven transmembrane α-helices with loops connecting them, being the N-terminal extracellular and C-terminal facing the intracellular side. Binding with a given substance leads to a conformational change in the receptor, leading to activation of the G protein docked on receptor's intracellular side, which initiates the specific cellular signaling process[14,15].

Following the International Union of Pharmacological Sciences taxonomic compatibility goals, it is possible to adopt a classification (to some extent minimalist, but widely accepted) of GPCR ligands that groups them into four categories according to their pharmacodynamic profile: agonists, antagonists, partial agonists, and inverse agonists.

In summary, agonists bind to receptors and activate the cellular response through conformational change. Antagonists bind to receptors and prevent the agonists from binding, generating no cellular response. The partial agonists works as a middle ground, binding to the receptors and generating an incomplete conformational response, but still allowing some cellular response, but blocking the receptors, preventing the full agonists from acting. So, ultimately, when both full and partial agonists are present, the partial agonists acts as competitive antagonists, decreasing the overall vector of receptor activation. The fourth group is represented by the inverse agonists, which induce a physiological response in the opposite direction to that expected from an agonist[12].

Although the idea that activation of a receptor only occurs when an agonist molecule binds to it is being spread, it is possible to find many examples that an appreciable level of activation can occur even in the absence of ligands[16].

Naturally occurring receptors or those that have undergone mutations (spontaneous or induced) can cause activation scenarios in the absence of a ligand, that is, constitutive activation. The occurrence of such activation without agonist binding is found in studies of G-protein-coupled receptors, such as cannabinoids[16,17].

Most of the time, constitutive receptor activation does not present magnitude for clinical repercussion, however, in certain conditions in which a large increase in receptor expression occurs, there may be pathophysiological implications of relevance. Plenty of scientific documentation of this is shown in studies on receptors for beta-adrenoreceptors and receptors for cannabinoids[16-18]. Evidence accumulated over the last three decades has suggested a two-state model[19] in which receptors are in equilibrium between an inactive conformation (R) and a spontaneously active conformation (R*) that can couple to G-protein in the absence of ligands.

Classical agonists have high affinity for R* and increase R* concentration, while inverse agonists have high affinity for R and decrease R* concentration. Neutral competitive antagonists have equal affinity for R and R* and do not shift the equilibrium, but can competitively antagonize the effects of both agonists and inverse agonists.

The concept of a two-state model is important for comprehending the basic mechanisms of action in various classes of drugs, but it does not correspond to reality. The receptors are not restricted to these two options, possessing conformational flexibility and more numerous possibilities. The different conformations that receptors are capable of adopting can be preferentially stabilized by different ligands and can produce different functional effects by activating different signal transduction pathways. The most current redefinition suggests a more complex scheme that contemplates a multi-state model and constitutes a challenge in this area of study. A given G-protein-coupled receptor such as cannabinoid can generate a diverse range of signaling responses, highlighting the physiological and clinical relevance of this class of proteins[20,21]. It is important to note that the pharmacodynamic role is independent of the ligand's affinity to the receptor. For example, it is possible to have complete agonists with weak binding and partial agonists with strong affinity.

The conformational change generated by ligand-receptor binding leads to a change in the relative orientation of transmembrane portions 3 (TM3) and 6 (TM6), which leads to the exposure of G-protein complex binding sites previously hidden on the intracellular side[22,23]. The heterotrimeric G-protein complex is specific for a particular type of GPCR, which once activated leads to inhibition or activation of various effector enzymes or ion channels.

The molecular structure of cannabinoid receptors comprehension has increased with recent studies of their crystallization[24-29]. To date, only the synthetic cannabinoid receptor-ligand set has been crystallized[12]. The structures of human CB1 and CB2 re-

ceptors share an aminoacid similarity of approximately 44% and a 68% homology with respect to transmembrane helices (TM)[5,30]. It has been shown that the binding site for the cannabinoid receptor is located in the membrane's lipid bilayer, with action on the receptor through lateral insertion of the ligand, rather than directly from the outer side, through solution[12,31]. The main differences between both receptors reside in the sequences of second extracellular N-terminal loop, TM7 C-terminal helix and intracellular C-termination itself[29,32]. These structural differences are precisely what confer preference for a given ligand.

CB1 receptor is preferentially found in the central nervous system (CNS), being more expressed in the presynaptic axon termination of several structures (amygdala, hippocampus, cortex, cerebellum, and basal ganglia circuitry)[12,33-35], being strongly associated with GABAergic and glutamatergic neurons[34]. Its activation ultimately leads to increased activity of potassium and calcium ion channels, which leads to the belief that its action is to modulate neurotransmitter release in a dependent manner[12].

Despite its predominance in CNS, the CB1 receptor is also found in the peripheral nervous system (PNS), especially in sympathetic fibers[36] and in nociceptors, notably in the dorsal root ganglia, trigeminal and dermal peripheral nerve endings, where it acts by regulating nociceptive afference[37-39].

In turn, CB2 receptor is strongly related to the immune system, with its activation being associated with neuronal defense mechanisms and inflammation reduction[40]. CB2 receptors are expressed mainly in the CNS, immune system cells, astrocytes, and mycroglia[40]. Besides its presence as a membrane receptor in these locations, it has been described the intracellular presence of CB2 receptor in prefrontal cortex pyramidal neurons in murine model, exerting modulation of neuronal excitability through Ca^{2+}-activated Cl^- channels[41,42], reinforcing that although its predominant expression is in the periphery, CB2R also has a role in neurological functions such as nociception, drug dependence, and neuroinflammation[43,44]. Although its presence in the CNS is up to 200 times less frequent than the CB1 receptor, there is an increase in its receptor transcription in situations of neurological insult such as chronic pain, stroke, and neuroinflammation[45,46].

As mentioned, the activity of both receptors, CB1 and CB2, is closely linked to the specific activation of G protein subunits. Classically, both receptors lead to suppression of adenylyl cyclase (AC) via $G_{i/o}$ signaling, which results in reduced levels of cyclic AMP (cAMP)[9,33,35,47]. However, as recently shown by a study[25], a difference in only one residue of the second intracellular loop (L222 in CB1 and P139 in CB2) may lead to coupling diversity between the cannabinoid receptor and the G protein family, with CB2 adopting a specificity only for G_i (conferred by the presence of the P138-P139 pattern in ICL2, unique to CB2)[24], while CB1 can vary between G_i, G_s, and G_q. Thus, an explanation arises for certain experimental findings, in which, under certain circumstances (for example, when there is concomitant dopaminergic activation in striatal neuron cultures), there was AC stimulation by G_s subunit after CB1 activation, leading to an increase in cAMP[48]. Added to this already complex scenario is the fact that there are also multiple possibilities of association between CB1 (through the $G_{\beta\gamma}$ subunit) and AC isoforms, generating predominance of stimulation (isoforms 2, 4, and 7) *versus* inhibition (1, 3, 5, 6, and 8)[9,49].

In addition to the orthosteric ligands, there is among the GPCR family receptors a modulation characteristic that allows them to broaden the spectrum of possibilities of conformational states and, therefore, of activation of intracellular signaling pathways: the interaction with *allosteric* ligands. Allosteric binding sites are those present in the receptor macromolecule, spatially distinct and not overlapping the so-called orthosteric site, but conformationally linked to it[50]. Allosteric modulators, when binding to the receptor in the absence of orthosteric ligand, can stimulate or inhibit the basal activity of this receptor, which was called *allo-agonism* and *allo-antagonism*, respectively. On the other hand, in the presence of the orthosteric ligand, allosteric modulation can alter the binding affinity of the former, as well as its efficiency in intracellular signaling[51].

Three features make these modulators especially interesting and potentially more effective than orthosteric binding: specificity, selectivity, and saturability[30,52-54]. Specificity is conferred by the greater frequency of variation in the aminoacid sequence making up the allosteric binding site (compared to the relative conservation in orthosteric domain sequence) and is thought to be the most important feature[52].

Selectivity in the target organ action is another relevant aspect. While the orthosteric ligand mostly affects receptor's signaling cascades in all tissues where it occurs, the allosteric modulation occurs mostly only in the tissue where the endogenous ligand was expressed in response to a particular stimulus[53]. Finally, saturation confers a ceiling effect, with no additional modulation expected apart from a certain threshold concentration of allosteric ligand, protecting against overdose[55]. Such characteristics, combined with the fact that drugs in clinical use, acting in ECs and based primarily on the orthosteric action of ligands, such as Dronabinol® and Cesamet®, generate considerable adverse effects (especially of psychoaffective order), have made the study on cannabinoid receptors' allosteric modulators an alternative for therapeutic application[52].

Inside the ECs, some ligands have been described as possessing allosteric modulatory activity. Lipoxin-4 (LXA4), an oxygenated derivative of AA, appears to act as a positive modulator of the CB1 receptor by strengthening anandamide affinity and activity[56]. Similarly, cholesterol and possibly other endogenous steroid derivatives such as pregnenolone have been verified in experimental models as possessing modulatory activity[25,57]. Some other endogenous allosteric modulators appear to exhibit positive function (PAM) for CB2 receptor and negative function (NAM) for CB1 receptor. Such is the case of pepcans (formerly hemopressins, endogenous cannabinoid peptides)[58,59].

The ECs, however, appears to have a much greater complexity than that dichotomized by these two receptors. Some authors have divided the receptors that bind to endogenous cannabinoids into three categories[60]: 1) receptors with extracellular binding site, represented mostly by GPCRs (such as the aforementioned CB1 and CB2); 2) receptors with intracellular EC binding site, such as those of transient receptor potential (TRP) family and 3) transcription factors, such as peroxisome proliferator-activated receptor (PPAR).

Besides the already studied GPCR, CB1 and CB2, it is worth mentioning that other receptors have been shown to have activation after binding with cannabinoids. It has been postulated that the orphan receptor GPR55 is a cannabinoid receptor, with authors already proposing its denomination as "CB3"[33,61,62]. The signaling pathway of this receptor involves multiple second messengers, which ultimately lead to increased intracellular Ca^{2+}. Interestingly, 2-AG exerts up to 200-fold greater potency as an agonist of GPR55 compared to its binding with the prototypical receptors (CB1 and CB2)[33,62]. However, these findings are not unanimous, with some authors not reproducing what has been found previously, failing to demonstrate ECs as activators of GPR55[61,63]. Thus, a more complete characterization of this receptor, with respect to its tissue distribution, subcellular localization, temporal pattern of expression, and the intracellular signaling pathways, is needed to lead to a greater comprehension of the ECs. Another orphan receptor that has also been listed as a possible cannabinoid receptor in the gastrointestinal tract is GPR119[64].

Robust evidence has been accumulated on the interaction between cannabinoids and transient receptor potential ion channels[33,60,65]. TRP receptors superfamily currently contains 28 known channels in mammals, subdivided into six subfamilies[66]. Among them, six channels (TRPV1-4, TRPA1 and TRPM8) have been shown to bind to cannabinoid substances (synthetic, vegetal and endocannabinoids), and they have been called *ionotropic cannabinoid receptors*[65]. These receptors are nothing more than true transmembrane pores, formed by tetramers (homo- or heteromerized). Each tetrameric subunit contains six transmembrane helices (S1-S6) that, when united, form an ion channel capable of regulating the entry of various cations in response to a stimulus[67]. When the action of ECs on these receptors was refined, so far only TRPV1, TRPV4, and TRPA1 showed consistent activation by endogenous ligands[65]. Anandamide has similar affinity to capsaicin in binding to TRPV1, but with less potent effect[68].

In 2003, a study showed activation of TRPV4 by prototypical ECs anandamide and 2-AG, being followed by other studies on the action of endogenous lipids such as N-acyl tryptophan and N-acyl tyrosine[69,70]. As for TRPA1, anandamide obtained a highly effective agonist action, about 59% higher than its prototypical agonist, mustard oil; TRPA1 was also activated by 2-AG[71]. In turn, TRPM8 seems to undergo antagonist action by anandamide[72]. It is due to the strong presence of these receptors (such as TRPV1 and TRPA1) in dorsal root nociceptor ganglia, the functional and clinical knowledge of their activation and the analgesic effect generated (such as application of topical capsaicin, for example) that the development of cannabinoid drugs for application in the treatment of chronic pain has been sought.

As a mechanism of action, it has been proposed that the modulation of these receptors by cannabinoids leads to immediate neuronal depolarization, followed subsequently by desensitization of these ion channels, which will remain in a silenced state, insensitive to the action of their ligands or thermal stimulation, which would precipitate a nociceptive stimulus[33]. Finally, PPARs are a family of heterodimeric nuclear hormone receptors, with three isoforms currently described (α, γ, and δ), which, when activated, bind to a DNA sequence (regions called *PPAR response elements*), leading to changes in the transcription of certain genes[73]. These target genes are listed in the regulation of metabolism, homeostasis, cell differentiation, and inflammation[74-76].

Since the 2000s, studies have shown that cannabinoid substances, among them ECs, bind to and activate such receptors[77]. Oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) activate PPARα, while anandamide and 2-AG also seem to show activity, although with less evidence, on α isoform and, more consistently, on γ isoform[78]. The activation of these receptors by PEA seems to exert an analgesic function *in vivo*, as has been observed in animal models of nociceptive behavior, either by testing PPARα inhibition through an antagonist or in knockout models[79-81]. However, the individual participation of these receptors in analgesia remains to be elucidated, as some authors find effects involving multiple receptors. One study, for example, identified that the analgesic effects of PEA on neuropathic pain involved CB1, TRPV1, and PPARγ receptors, but not its α isoform or CB2R[82].

THE ENDOCANNABINOID PROCESSING MACHINERY AND ITS RELATIONSHIPS INSIDE THE ENDOGENOUS CANNABINOID SYSTEM

ECs are signaling lipid molecules comprised of two major groups: N-acylethanolamines (NAE) and monoacylglycerols (MAG)[11]. As mentioned, the two most studied ECs so far are anandamide and 2-AG, presenting different pharmacological characteristics. While anandamide seems to behave as a high-affinity partial agonist of the CB1 receptor, being almost inactive in CB2, 2-AG acts as a full agonist in both, but with low to moderate affinity[9,10,83]. Both are produced on demand, but synthesis, transport and inactivation occur differently according to the target tissue[9]. The basal levels of 2-AG are up to a thousand times higher than those of anandamide in the brain. Experimental studies that manipulated 2-AG metabolism (but not anandamide) had marked effects on endocannabinoid retrograde signaling. Thus, a consensus has been reached that 2-AG is the primary endogenous ligand of cannabinoid receptors in CNS[9-11,84,85].

As stated, ECs are produced on demand, and it should be kept in mind that they have a short half-life (approximately 15 minutes) and that metabolic enzymes and carrier molecules are responsible for their delivery to the target receptor in the exact and precise concentration[60]. Redundancy is a hallmark of the endocannabinoid biosynthesis and degradation system, with several pathways -including those that are responsible for the synthesis of other NAE and MAG – resulting in anandamide and 2-AG production[86,87]. Two enzymes, however, stand out: anandamide has N-acyl-phosphatidylethanolamine (NAPE) as its precursor form, synthesized by the enzyme NAPE-specific phospholipase D (NAPE-PLD)[9,88]; in turn, 2-AG is produced from diacylglycerol (DAG), by DAG lipases (DAGL) α or β – with studies evidencing that virtually all 2-AG involved in

adult brain's synaptic transmission is formed by DAGL α[9,85]. However, the limiting step in production of both is the formation of NAPE and DAG, which are converted from phosphatidylethanolamine by N-acyltransferase, and from phosphoinositides by phospholipase C, respectively[9,85,88].

Once synthesized and released into cytosol, ECs are unable to diffuse freely like other neurotransmitters, due to their hydrophobic nature. Thus, several mechanisms such as binding to certain carrier proteins, as well as endocytosis through the use of lipid "rafts"/caveolae have been studied and proposed as a means to transport anandamide and 2-AG, the latter being less elucidated, but probably sharing the system used by the former[9]. Heat shock protein (HSP) 70, albumin, fatty acid-binding proteins (FABPs) 5 and 7, and albumin itself have been listed[89-91]. As for the transport in extracellular medium, more specifically in the synaptic cleft, it seems to occur in microvesicles, instead of the transport occurring through a binding with transport proteins[92,93].

The ECs, as already pointed out, acts primarily as a suppressor of synaptic activity, regardless of the nature of the synapse or the transmission duration[89,94]. In most cases, endocannabinoid retrograde signaling starts with 2-AG production in postsynaptic neurons, in response to the increase of intracellular Ca^{2+} or of receptors bound to $G_{q/11}$ unit. Transport across the synaptic cleft then occurs, and EC binds to CB1R located on the presynaptic membrane. In turn, the activated CB1R suppresses neurotransmitter release by two main mechanisms: 1) by inhibiting voltage-dependent Ca^{2+} channels, thus decreasing the influx of presynaptic signaling cation; 2) by inhibiting AC and the subsequent cAMP/PKA pathway, which is involved in long-term depression (LTD)[89,94,95].

Anandamide also acts in a retrograde manner, but via multiple mechanisms, the main one being through TRPV1[96] receptors. The localization of the enzymes that synthesize ECs plays a crucial role in this context and seems to be associated with lipid sites inside the plasma membrane, called "rafts". The enzymatic machinery responsible for 2-AG production, for example, seems to concentrate in these microdomains[97]. These rafts also act effectively in AEA reuptake, as well as in the recycling of its metabolites, AA and ethanolamine, which are found in concentrated form in these membrane portions.

Anandamide is metabolized primarily by fatty acid amide hydrolase (FAAH), located mainly in postsynaptic neuron endoplasmic reticulum[98,99]. This enzyme also catabolizes other N-acylethanolamines, such as PEA and OEA, which despite having little biological activity on CB1 and CB2 receptors, can raise AEA levels indirectly, by competing as substrate for FAAH[100,101]. As degradation metabolites of anandamide, the aforementioned AA and ethanolamine remain. In turn, 2-AG is catabolized into AA and glycerol by monoacylglycerol lipase (MGL or MAGL), present in the presynaptic neuron[102,103]. Multiple other enzymes are also listed, such as FAAH itself[104,105] and enzymes in α/β hydrolases domain, such as ABHD2[106], 4[60], 6[107], and 12[108]. ECs can also undergo oxidation by AA cascade enzymes, such as cyclooxygenase 2 (COX-2) and by various lipoxygenases (LOXs)[109], with their oxidative by-products possessing their own biological activities in ECs, distinct from the ECs that generated them[110].

Based on the above it is clear that understanding lipid metabolism is fundamental to a complete ECs understanding. It is even more important to remember that there is a high diversity in the lipid membranes of eukaryotes[111] and that a large part of the enzymes belonging to ECs are membrane-bound proteins. Their activities and availability in the membrane can be affected by different lipids in the vicinity. In the case of FAAH, for example, it has been shown that cholesterol present in the membrane is responsible for stabilizing a dimeric form of the enzyme, as well as modulating its localization at subcellular level (i.e., in organelle membranes), and increasing its catalytic activity, which ultimately affects the extent to which EC signaling is propagated at the intracellular level and consequently its termination[112].

Similarly, the study of acyl chains composition in plasma membranes has gained relevance, demonstrating that the length and saturation degree of chains are crucial for intra and transmembrane trafficking and enzyme degradation processes[113]. Thus, although MAGL can hydrolyze several monoacylglycerols – all containing the same glycerol pole as 2-AG, but with distinct acyl chains – it is the length and saturation of their chains that will define the speed of hydrolysis rate, being up to 2x faster for 2-AG (longer and polyunsaturated chain) compared to its congener 2-PG (2-palmitoylglycerol, shorter and saturated chain)[113].

Interestingly, it has recently been shown that ABHD2 activity is progesterone-dependent in sperm, in which 2-AG acts as an endogenous inhibitor of a cation channel known as CatSper. In the presence of said hormone, this enzyme hydrolyzes 2-AG and leads to the CatSper channels opening, hyperactivating and ultimately making the sperm fertile[106]. The finding that the level of 2-AG is controlled by the stimulation of its degradation is of great relevance, since it casts questions on the current dogma of "production on demand" of the ECs, i.e., that ECs are produced only by controlling their biosynthesis in a stimulus-dependent manner from phospholipid precursors. At least in semen, 2-AG is "hydrolyzed on demand" from a preexisting pool[106] and finally adjusted by steroid hormones.

These examples show the ECs complexity, since the same cannabinoid receptor (e.g. CB1) or metabolic enzyme (e.g. FAAH), within the same cell, but under different lipid conditions, can culminate in different EC signaling and lead to different biological behaviors[60].

CONCLUSION

The ECs components are widely expressed in different tissues and compose a lipid signaling system, playing a key role in the regulation of several physiological processes such as metabolism, mood, appetite, cardiovascular control, motor function, immune system, neurotransmission and nociception. The comprehension of its elements and a better understanding of receptors and enzymes ultrastructure will decisively contribute to the development of new pharmacological strategies that are not limited to CB1R

direct action, for example. Of the six enzymes involved in 2-AG metabolization, for example, only the MAGL structure is known. From the detailed description of the machinery responsible for endocannabinoid lipid metabolization, it will be possible to unlock the potential for the development of new drugs (such as analgesics without the CB1-mediated adverse effects) and their translation into clinical practice.

AUTHORS' CONTRIBUTIONS

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Data Collection, Conceptualization, Writing - Preparation of the Original
Leonardo Rafael Takahashi
Writing - Review and Editing
José Oswaldo de Oliveira Junior
Conceptualization, Writing - Review and Editing , Supervision

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