The cannabinoids mechanism of action: an overview

Mecanismo de ação dos canabinoides: visão geral

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ABSTRACT

BACKGROUND AND OBJECTIVES: The discovery of the psychoactive agent of *Cannabis sativa* (tetrahydrocannabinol - THC) in the second half of the 20th century originated the research that later came to identify dozens of other substances from this plant, including cannabinoids, terpenes and flavonoids. Ensuing description of their interaction sites in animals and humans, together with endogenous ligands, transport proteins as well as synthesis and degradation enzymes, revealed what came to be known as the endocannabinoid system. Several receptors participate in this system.

CONTENTS: The first receptors to be discovered were called CB1 and CB2, both are G protein-coupled (GPCR). It is noteworthy that CB1 receptors are among the most abundant and widely distributed GPCR in the mammalian brain, with marked expression in basal ganglia, cerebellum and hippocampus, for instance; on the other hand, they are scarce in areas of the brainstem related to breathing control. In light of the multiplicity of pharmacological effects of cannabinoids, concomitant with the lack of more clarifying studies on their mechanisms of action despite the great interest in research on their therapeutic application, it is necessary to deepen the knowledge in this area.

CONCLUSION: Considering the literature research conducted for the composition of this article, it is possible to conclude that cannabinoids have a broad spectrum of action mechanisms in the human body, and that more robust clinical studies are needed to better understand their broad therapeutic potential.

Keywords: Cannabis, Cannabinoid receptor agonists, Cannabinoid receptor antagonists, Cannabinoids, Modulators, Neurobiology.

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HIGHLIGHTS
• Knowledge of the endocannabinoid system allows for a better comprehension of its beneficial effects.
• Clinical efficacy, positive or otherwise, is very much related to the functioning of this system of neurotransmitters, receptors, and enzymes.

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RESUMO

JUSTIFICATIVA E OBJETIVOS: A descoberta do princípio psicoativo da *Cannabis sativa* (tetrahidrocanabinol - THC) na segunda metade do século XX inaugurou pesquisas que posteriormente vieram a identificar dezenas de outras substâncias a partir dessa planta, incluindo canabinoides, terpenos e flavonoides. A subsequente descrição dos sítios de interação dessas substâncias em animais e humanos, assim como seus ligantes endógenos, proteínas de transporte e enzimas de síntese e degradação, revelou o que veio a ser conhecido como sistema endocanabinoide. Diversos receptores participam deste sistema.

CONTEÚDO: Os primeiros receptores a serem descobertos foram denominados CB1 e CB2, ambos são acoplados à proteína G (GPCR). É importante ressaltar que os receptores CB1 estão entre os GPCRs mais abundantes e amplamente distribuídos do encéfalo de mamíferos, com marcada expressão, por exemplo, em gânglios da base, cerebelo e hipocampo; em contrapartida, são escassos em áreas do tronco cerebral relacionadas ao controle da respiração. Diante da multiplicidade de efeitos farmacológicos dos canabinoides, concomitante à falta de estudos mais esclarecedores sobre seus mecanismos de ação apesar do grande interesse na pesquisa de sua aplicação terapêutica, é preciso aprofundar o conhecimento nessa área.

CONCLUSÃO: Considerando as pesquisas bibliográficas realizadas para a composição deste artigo, é possível concluir que os canabinoides possuem um amplo espectro de mecanismos de ação no organismo humano, e que mais estudos clínicos robustos são necessários para que seja possível entender melhor o seu amplo potencial terapêutico.

Descritores: Agonistas de receptores de canabinoides, Antagonistas de receptores de canabinoides, Cannabis, Canabinoides, Moduladores, Neurobiologia.

INTRODUCTION

The discovery of *Cannabis sativa* psychoactive principle (tetrahydrocannabinol - THC) in the second half of the 20th century inaugurated research that later came to identify dozens of other substances from this plant, including cannabinoids, terpenes, and flavonoids[1,2]. The subsequent description of interaction sites for these substances in animals (most vertebrates, such as dogs,

cats, horses, rabbits, and others) and humans, as well as their endogenous ligands, transport proteins, and synthesis and degradation enzymes, revealed what has come to be known as the endocannabinoid system[1].

Several receptors participate in this system. The first to be discovered were denominated CB1 and CB2, both of which are G protein-coupled receptors (GPCR)[3]. Therefore, they have extracellular binding sites and an intracellular guanine nucleotide binding protein. It is important to note that CB1 receptors are among the most abundant and widely distributed GPCRs in the mammalian brain[4,5], with marked expression, for example, in basal ganglia, cerebellum and hippocampus[6]; on the other hand, they are scarce in brainstem areas related to respiration control[5]. CB1s, on the other hand, have significant expression in the medulla and peripheral nerve endings[6], and have been identified at other sites, such as the cardiovascular system[7], gastrointestinal tract[8] and liver[9]. In turn, CB2 receptors are known to predominate in the immune system, especially in cells of macrophage origin, including microglia[3], but are also present in lower expression in other tissues such as bone, reproductive system, cardiovascular, gastrointestinal[10] and brain[11]. Interestingly, some receptors also coupled to G protein with extracellular binding site, but with unknown endogenous ligands (hence being called orphan receptors - GPR), are also part of the system in question by having affinity for cannabinoids[12].

Also included in the endocannabinoid system are receptors with intracellular binding sites, the most important of which are the ionotropic vanilloid receptors (transient receptor potential - TRPV)[13] and transcription factors such as the nuclear peroxisome proliferator-activated receptors (PPAR)[14].

The main endogenous molecules that interact with cannabinoid receptors are anandamide (AEA) and 2-araquinodoylglycerol (2-AG)[15-17]. The effects of this interaction depend on the availability of these endocannabinoid substances in the cell, which, in turn, results from the balance between their synthesis and degradation[2]. Both come from enzymatic activity on phospholipids. AEA can be synthesized by n-acetyltransferase (NAT), n-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD), glicero-phosphodiesterases[18] and alpha/beta hydrolase[19]. The degradation of AEA is due to fatty acid amide hydrolase (FAAH) and n-acylethanolamine acid amidase (NAAA)[2,20]. In turn, 2-AG is produced by diacylglycerol lipases alpha and beta (DAGL) and inactivated by monoacylglycerol lipase (MAGL)[2,20] and alpha/beta-beta hydrolases[19].

This set of reactions needs to be highly precise, since endocannabinoids have a short half-life, approximately 15 minutes[2]. Indeed, it is classically recognized that these substances are produced and released on demand. However, there is evidence that these processes are at least complemented by intracellular transport and storage[21]. This makes sense insofar as endocannabinoids participate both in extracellular signaling and at all levels of the intracellular space. Furthermore, these substances are lipidic, made to travel through the aqueous environments of cytosol and extracellular space, depending on carrier molecules[21]. The knowledge about these molecules is still rudimentary, and a chaperone (HSP70), albumin, and fatty acid-binding proteins (FABP)[22,23],

which are intracellular transporters, have been identified; there is a membrane transporter (EMT)[24] and in the extracellular compartment this movement occurs through microvesicles[2].

Notably, the kinetics and dynamics of endocannabinoids reveal that they are predominantly retrograde neurotransmitters[25,26]. Thus, their synthesis occurs in response to depolarization and increased calcium levels in postsynaptic cells, from where they are transported across the synaptic cleft to presynaptic cells[25-27]. This model is interesting in the regulation of nociceptive pathways, so that the occurrence of a noxious stimulus transmitted to the postsynaptic neuron would cause it to synthesize and release endocannabinoids. These would reach presynaptic neurons by activating and inducing the expression of CB1 receptors, which, in turn, inhibit voltage-gated calcium channels and activate potassium channels. The result is restriction of the excitatory neurotransmitters release[28,29]. The activation of cannabinoid receptors, also via G protein coupled, determines the inhibition of enzyme adenylyl cyclase and synthesis of cyclic AMP, with consequent suppression of protein kinase A activity, an important intracellular signaling pathway in the production of energy through glycogenolysis and lipid metabolism[27].

This set of data indicates that cannabinoid system is widely involved in the suppression of neurotransmission and excitability, which is essential to control pathological states of excitotoxicity, such as chronic pain[29]. This is in line with the evidence of this system participation in short- and long-term neuroplasticity[30].

Finally, it is relevant to consider that the significant presence of CB1 receptors in areas essential to pain control, such as cortex, limbic system[31], periaqueductal gray, rostral ventromedial medulla[32], posterior grey column[33], trigeminal ganglion[34], dorsal root ganglia, and peripheral nerves[35], corroborates the endocannabinoid system importance in potential modification of somatic and affective aspects of pain[29].

Interestingly, cannabinoid receptors are able to interact with different forms of G-protein, leading to the activation of other important intracellular signaling pathways. Such is the case with mitogen-activated protein kinases (MAPKs), this diverse class of enzyme pathways is associated with cell proliferation, cell cycle and cell death. Its relationship with the cannabinoid system is of such complexity that depending on intracellular environment and the ligands involved, it can result in cell survival or death[36]. There is still much to be known about the intracellular signaling pathways of cannabinoids, but it is already known that CB1 receptors also exist in this space, either by internalization of receptors originally located in cell membrane[37], or as part of a desensitization process in the interaction pathway with beta-arrestin independent of G protein[38], and finally, as receptors of diverse functionalities and originally expressed inside the cell[38]. There is evidence, for example, of CB1 receptors in lysosomes, paradoxically associated with an intracellular increase of calcium[39], as well as in mitochondria, where they determine the reduction of cyclic AMP, mitochondrial respiration, neuroprotection, appetite and memory, according to studies on hypothalamic neurons[40].

At the same time that its wide distribution in nervous system indicates that CB1 can have several therapeutic applications, this same characteristic tends to restrict its clinical application by the poten-

tial for adverse effects[38]. Although the distribution of CB1 receptors is very wide in central nervous system (CNS), leading to the think that it would have several therapeutic applications, however, this anatomical property leads to restrict its clinical application because it generates a significant potential for adverse effects, even though the expression of this receptor can oscillate in pathological states[41]. However, this restriction seems to be much smaller in the case of the CB2 receptor, whose expression is much more demand-dependent, so that tissues affected by diseases would be activated with greater selectivity[42]. Although this characteristic is advantageous in therapeutic terms, it constitutes a significant obstacle in the study of localization, structure, and function of these receptors. Despite advances in this understanding through studies with active and inactive models of these receptors, more details about the activation mechanisms of CB2 are still unclear[43].

Knowing that they are predominant in immune cells and other systems such as cardiovascular and digestive already indicates their potential for therapy, but more recently, CB2 receptor has also been identified in nervous system and studies point to its participation in pain modulation and neuroinflammation[10]. This is reinforced by the wide presence of cannabinoid receptors in glial cells as well, whose importance is increasingly recognized in the excitotoxicity present especially in neurodegenerative diseases[44].

The complexity of cannabinoid receptors also extends to their relationships with ligands. These can induce variable expressions of receptors, which can present a surprising multiplicity of conformations that, in turn, will lead to the activation of diverse signaling pathways[42]. Thus, there is a functional selectivity with great variability in affinity and response to different ligands. This makes the definition, research and development of agonists and antagonists complicated.

Studies on signaling of the endocannabinoid system suggest that 2-AG is probably the primary endogenous ligand of cannabinoid receptors, which is in line with the fact that this ligand has levels 1000 times higher than AEA in the brain[38]. These substances also differ widely in terms of their relationship to the main receptors in this system. Traditionally, 2-AG is considered to act as a moderate affinity agonist for CB1 and CB2, whereas AEA is a partial agonist for CB1 and basically inactive for CB2[45]. This does not mean that AEA is irrelevant, since it also acts on cannabinoid system, especially on ion channels via type 1 vanilloid receptors (TRPV1), and it can also act in an anterograde way[46].

Besides endocannabinoids, there are more than 100 substances derived from cannabis plant capable of interacting with cannabinoid receptors, which are the phytocannabinoids, among which tetrahydrocannabinol (THC) and cannabidiol (CBD) stand out. Tetrahydrocannabinol was the first phytocannabinoid to be discovered. It is the major responsible for cannabis psychoactive effects, through its agonist action on CB1 receptors and consequent interference in GABA/glutamate balance[47]. It also causes release of dopamine with striato-limbic selectivity, although at lower levels than other substances of abuse, such as amphetamine and nicotine, as was demonstrated by a study using positron emission tomography[48]. The wide distribution of CB1 in nervous system is compatible with the observation of multiple effects of THC.

One of the most important, known and interesting from a therapeutic point of view is certainly its antiemetic action. This is mainly due to the important expression of CB1 receptors in the dorsal vagal complex. However, there is evidence that central serotoninergic (5HT3) and vanilloid (TRPV1) receptors, as well as peripheral CB1s also participate[49]. THC also has analgesic potential, both through CB1 receptors, whose presence in key sites of pain transmission and processing in CNS has already been cited; and through agonist action on CB2 receptors with anti-inflammatory results[47]. However, the clinical use of THC is commonly limited by its psychoactive effects, including euphoria, anxiety, and even psychosis[50].

This is not the case with CBD, a phytocannabinoid discovered a few years after THC, which curiously acts as an antagonist for CB1 and CB2[51] receptors, which offers an advantage not only by not giving it the adverse cognitive-behavioral effects of THC over CB1, but by being able to attenuate them[52] when these substances are used in preparations that combine them. There is evidence that CBD acts on several other receptors. Its antiemetic, anxiolytic, and analgesic actions, at least in part, can be mediated by binding to serotonergic receptors, especially 5HT1A[47,53]. This same pathway would be involved in the reduction of excitotoxicity, oxidative stress, and proinflammatory activation, including microglial and lymphocytic.

Such effect would be compatible with the possible protective action of CBD in several diseases, such as neurodegenerative diseases (including Parkinson's disease and Alzheimer's disease), chronic pain, inflammatory, cardiac, hepatic, renal and gastrointestinal diseases, sepsis, and diabetes[53]. One of the first applications discovered for CBD was its ability to contribute significantly to alleviating the suffering of patients with difficult to control epilepsy[54]. An attempt is still being made to unravel the underlying mechanisms. Besides the action on 5HT1 receptors, it has been postulated that there is CBD activity on gabaergic receptors and ion channels[53].

Also of great interest is the possible contribution of CBD to antineoplastic therapy. At higher concentrations, it has been found to inhibit the proliferation of human cancer cells of various lineages, such as prostate, breast and colorectal[53]. Several ways to explain this result have been suggested, including the participation of vanilloid receptors (TRPV1), CB2 and PPAR, compatible with the great pharmacological versatility of this phytocannabinoid[53]. Still in this sense, there is evidence of CBD interaction with mu and delta opioid receptors, which, in association with its affinity for vanilloid receptors, reinforces its application in pain treatment[47,55].

More recently, another phytocannabinoid has gained attention, cannabigerol (CBG), which has an intermediate pharmacological profile between THC and CBD. It shows agonism for both CB1 and CB2, but with much lower affinity than THC, and at the same time, it is able to associate with several vanilloid receptors (TRPV) like CBD, also with lower affinity[56]. However, there are some peculiarities; CBG is a potent agonist of alpha-2 adrenergic receptors[56]. The importance of these in blood pressure modulation, sedation and analgesia is well known. Its extensive distribution in the nervous system, with special emphasis on its

participation in modulating prefrontal cortex activity, has attracted much interest about the use of alpha-2 adrenergic agonists in treatments for neuropsychiatric disorders, including attention deficit hyperactivity disorder[57]. Another uniqueness of CBG is its antagonism to the 5HT1 receptor, unlike CBD and THC[56]. All this considered, there is a need to better understand the interaction of CBG with the different subfamilies of the wide variety of receptors it is related to in order to safely elucidate its indications and limitations.

Such care, obviously, must be applied to the use of any group of substances. This is especially the case with cannabinoids, not only because the phytocannabinoids pharmacological diversity, of which there are more than a hundred, and of which only the best known are mentioned, but also because there are other groups of substances present in *Cannabis sativa*, such as the terpenes, hydrocarbons responsible for cannabis aroma, which also have therapeutic potential[58]. Apparently, these substances do not act through the CB1 and CB2 receptors[59], but studies showing that extracts of the plant have superior effects compared to isolated phytocannabinoids, strongly suggest the existence of an "entourage effect"[60,61]. This would correspond to a synergy between cannabis phytomolecules. If this occurs between phytocannabinoids or between terpenes, it is an intra-entourage effect, if on the other hand phytocannabinoids and terpenes participate in the synergy, it is an inter-entourage effect[58]. The pharmacological processes by which these phenomena may occur are not known, but they are extremely interesting as future possibilities.

CONCLUSION

The great complexity of the endocannabinoid system, as well as its relationships with exogenous cannabinoids, the several types of receptors and systems in such a wide and varied distribution in the body, give a measure of the fascinating challenge of exploring so many possibilities, which can benefit the health of many people.

AUTHORS' COLABORATIONS

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REFERENCES

1. Starowicz K, Finn DP. Cannabinoids and Pain: Sites and Mechanisms of Action. in: Advances in Pharmacology [Internet]. Elsevier; 2017 [citado 23 de junho de 2022]. p. 437–75. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S1054358917300443
2. Maccarrone M. Missing pieces to the endocannabinoid puzzle. Trends Mol Med. 2020;26(3):263-72.
3. Pertwee RG. The diverse CB₁ and CB₂ receptor pharmacology of three plant cannabinoids: Δ⁹-tetrahydrocannabinol, cannabidiol and Δ⁹-tetrahydrocannabivarin: Δ⁹-THC, CBD and Δ⁹-THCV. Br J Pharmacol. 2008;153(2):199-215.
4. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC. Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A. 1990;87(5):1932-6.
5. Mackie K. Cannabinoid receptors: where they are and what they do. J Neuroendocrinol. 2008;20(s1):10-4.
6. Hu SSJ, Mackie K. Distribution of the Endocannabinoid System in the Central Nervous System. In: Pertwee RG, organizador. Endocannabinoids [Internet]. Cham: Springer International Publishing; 2015 [citado 22 de julho de 2022]. 59-93p. (Handbook of Experimental Pharmacology; vol. 231). Disponível em: http://link.springer.com/10.1007/978-3-319-20825-1_3
7. Montecucco F, Di Marzo V. At the heart of the matter: the endocannabinoid system in cardiovascular function and dysfunction. Trends Pharmacol Sci. 2012;33(6):331-40.
8. Cohen L, Neuman MG. Cannabis and the gastrointestinal tract. J Pharm Pharm Sci. 2020;23:301-13.
9. González-Mariscal I, Krzysik-Walker SM, Doyle ME, Liu QR, Cimbro R, Santa-Cruz Calvo S, Ghosh S, Cieśla Ł, Moaddel R, Carlson OD, Witek RP, O'Connell JF, Egan JM. Human CB1 receptor isoforms, present in hepatocytes and β-cells, are involved in regulating metabolism. Sci Rep. 2016;6:33302.
10. Howlett AC. International Union of Pharmacology. XXVII. Classification of Cannabinoid Receptors. Pharmacol Rev. 2002;54(2):161-202.
11. den Boon FS, Chameau P, Schaafsma-Zhao Q, van Aken W, Bari M, Oddi S, Kruse CG, Maccarrone M, Wadman WJ, Werkman TR. Excitability of prefrontal cortical pyramidal neurons is modulated by activation of intracellular type-2 cannabinoid receptors. Proc Natl Acad Sci U S A. 2012;109(9):3534-9.
12. Irving A, Abdulrazzaq G, Chan SLF, Penman J, Harvey J, Alexander SPH. Cannabinoid receptor-related orphan g protein-coupled receptors. Adv Pharmacol. 2017;80:223-47.
13. Muller C, Morales P, Reggio PH. Cannabinoid ligands targeting TRP channels. Front Mol Neurosci. 2019;11:487.
14. O'Sullivan SE. An update on PPAR activation by cannabinoids: cannabinoids and PPARs. Br J Pharmacol. 2016;173(12):1899-910.
15. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258(5090):1946-9.
16. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol. 1995;50(1):83-90.
17. Suchopár Josef, Laštůvka Zdeněk, Mašková Simona, Alblová Miroslava, Pařízek Antonín. Endocannabinoids. Česka Gynekol. 2021;86(6):414-20.
18. Hussain Z, Uyama T, Tsuboi K, Ueda N. Mammalian enzymes responsible for the biosynthesis of N-acylethanolamines. Biochim Biophys Acta BBA. Mol Cell Biol Lipids. 2017;1862(12):1546-61.
19. Cao JK, Kaplan J, Stella N. ABHD6: its place in endocannabinoid signaling and beyond. Trends Pharmacol Sci. 2019;40(4):267-77.
20. Ligresti A, Cascio M, Marzo V. Endocannabinoid metabolic pathways and enzymes. Curr Drug Target -CNS Neurol Disord. 2005;4(6):615-23.
21. Maccarrone M, Dainese E, Oddi S. Intracellular trafficking of anandamide: new concepts for signaling. Trends Biochem Sci. 2010;35(11):601-8.
22. Oddi S, Fezza F, Pasquariello N, D'Agostino A, Catanzaro G, De Simone C, Rapino C, Finazzi-Agrò A, Maccarrone M. Molecular identification of albumin and Hsp70 as cytosolic anandamide-binding proteins. Chem Biol. 2009;16(6):624-32.
23. Kaczocha M, Glaser ST, Deutsch DG. Identification of intracellular carriers for the endocannabinoid anandamide. Proc Natl Acad Sci. 2009;106(15):6375-80.
24. Chicca A, Marazzi J, Nicolussi S, Gertsch J. Evidence for bidirectional endocannabinoid transport across cell membranes. J Biol Chem. 2012;287(41):34660-82.
25. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 2009;89(1):309-80.
26. Kreitzer AC, Regehr WG. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto purkinje cells. Neuron. 2001;29(3):717-27.
27. Ohno-Shosaku T, Kano M. Endocannabinoid-mediated retrograde modulation of synaptic transmission. Curr Opin Neurobiol. 2014;29:1-8.
28. Pertwee RG. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol Ther. 1997;74(2):129-80.
29. Woodhams SG, Sagar DR, Burston JJ, Chapman V. The role of the endocannabinoid system in pain. Handb Exp Pharmacol. 2015;227:119-43.
30. Chevaleyre V, Takahashi KA, Castillo PE. Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci. 2006;29(1):37-76.
31. Herkenham M, Lynn A, Johnson M, Melvin L, de Costa B, Rice K. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci. 1991;11(2):563-83.
32. Bouchet CA, Ingram SL. Cannabinoids in the descending pain modulatory circuit: role in inflammation. Pharmacol Ther. 2020;209:107495.
33. Hegyi Z, Kis G, Holló K, Ledent C, Antal M. Neuronal and glial localization of the cannabinoid-1 receptor in the superficial spinal dorsal horn of the rodent spinal cord. Eur J Neurosci. 2009;30(2):251-62.
34. Price TJ, Helesic G, Parghi D, Hargreaves KM, Flores CM. The neuronal distribution of cannabinoid receptor type 1 in the trigeminal ganglion of the rat. Neuroscience. 2003;120(1):155-62.
35. Veress G, Meszar Z, Muszil D, Avelino A, Matesz K, Mackie K, Nagy I. Characterisation of cannabinoid 1 receptor expression in the perikarya, and peripheral and spinal processes of primary sensory neurons. Brain Struct Funct. 2013;218(3):733-50.
36. Olianas MC, Dedoni S, Onali P. Cannabinoid CB1 and CB2 receptors differentially regulate TNF-α-induced apoptosis and LPA1-mediated pro-survival signaling in HT22 hippocampal cells. Life Sci. 2021;276:119407.

37. Leterrier C, Bonnard D, Carrel D, Rossier J, Lenkei Z. Constitutive endocytic cycle of the CB1 cannabinoid receptor. J Biol Chem. 2004;279(34):36013-21.

38. Zou S, Kumar U. Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. Int J Mol Sci. 2018;19(3):833.

39. Brailoiu GC, Oprea TI, Zhao P, Abood ME, Brailoiu E. intracellular cannabinoid type 1 (CB1) receptors are activated by anandamide. J Biol Chem. 2011;286(33):29166-74.

40. Bénard G, Massa F, Puente N, Lourenço J, Bellocchio L, Soria-Gómez E, Matias I, Delamarre A, Metna-Laurent M, Cannich A, Hebert-Chatelain E, Mulle C, Ortega-Gutiérrez S, Martín-Fontecha M, Klugmann M, Guggenhuber S, Lutz B, Gertsch J, Chaouloff F, López-Rodríguez ML, Grandes P, Rossignol R, Marsicano G. Mitochondrial CB1 receptors regulate neuronal energy metabolism. Nat Neurosci. 2012;15(4):558-64.

41. Miller LK, Devi LA. The Highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. Pharmacol Rev. 2011;63(3):461-70.

42. Atwood BK, Straiker A, Mackie K. CB2: therapeutic target-in-waiting. Prog Neuropsychopharmacol Biol Psychiatry. 2012;38(1):16-20.

43. Ye L, Cao Z, Wang W, Zhou N. New insights in cannabinoid receptor structure and signaling. Curr Mol Pharmacol. 2019;12(3):239-48.

44. Stella N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia. 2010;58(9):1017-30.

45. Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB$_1$ and CB$_2$. Pharmacol Rev. 2010;62(4):588-631.

46. Aguiar DC, Moreira FA, Terzian AL, Fogaça MV, Lisboa SF, Wotjak CT, Guimaraes FS. Modulation of defensive behavior by Transient Receptor Potential Vanilloid Type-1 (TRPV1) channels. Neurosci Biobehav Rev. 2014;46(Pt 3):418-28.

47. Boggs DL, Nguyen JD, Morgenson D, Taffe MA, Ranganathan M. Clinical and preclinical evidence for functional interactions of cannabidiol and Δ9-Tetrahydrocannabinol. Neuropsychopharmacology. 2018;43(1):142-54.

48. Bossong MG, Mehta MA, van Berckel BNM, Howes OD, Kahn RS, Stokes PRA. Further human evidence for striatal dopamine release induced by administration of Δ9-tetrahydrocannabinol (THC): selectivity to limbic striatum. Psychopharmacology (Berl). 2015;232(15):2723-9.

49. Parker LA, Rock EM, Limebeer CL. Regulation of nausea and vomiting by cannabinoids: cannabinoids and nausea and vomiting. Br J Pharmacol. 2011;163(7):1411-22.

50. Banister SD, Arnold JC, Connor M, Glass M, McGregor IS. Dark classics in chemical neuroscience: δ ^{9}-tetrahydrocannabinol. ACS Chem Neurosci. 2019;10(5):2160-75.

51. Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG. Cannabidiol displays unexpectedly high potency as an antagonist of CB$_1$ and CB$_2$ receptor agonists *in vitro*: Cannabinoid antagonism by cannabidiol. Br J Pharmacol. 2007;150(5):613-23.

52. Zhornitsky S, Potvin S. Cannabidiol in humans--the quest for therapeutic targets. Pharmaceuticals. 2012;5(5):529-52.

53. Pacher P, Kogan NM, Mechoulam R. Beyond THC and endocannabinoids. Annu Rev Pharmacol Toxicol. 2020;60(1):637-59.

54. Ligresti A, De Petrocellis L, Di Marzo V. From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. Physiol Rev. 2016;96(4):1593-659.

55. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci. 2009;30(10):515-27.

56. Nachnani R, Raup-Konsavage WM, Vrana KE. The pharmacological case for cannabigerol. J Pharmacol Exp Ther. 2021;376(2):204-12.

57. Arnsten AF. The use of α-2A adrenergic agonists for the treatment of attention-deficit/hyperactivity disorder. Expert Rev Neurother. 2010;10(10):1595-605.

58. Koltai H, Namdar D. Cannabis phytomolecule "entourage": from domestication to medical use. Trends Plant Sci. 2020;25(10):976-84.

59. Santiago M, Sachdev S, Arnold JC, McGregor IS, Connor M. Absence of entourage: terpenoids commonly found in *cannabis sativa* do not modulate the functional activity of Δ^9-THC at human CB$_1$ and CB$_2$ receptors. Cannabis Cannabinoid Res. 2019;4(3):165-76.

60. Gonçalves ECD, Baldasso GM, Bicca MA, Paes RS, Capasso R, Dutra RC. Terpenoids, cannabimimetic ligands, beyond the cannabis plant. Molecules. 2020;25(7):1567.

61. Russo EB. The case for the entourage effect and conventional breeding of clinical cannabis: no "strain," no gain. Front Plant Sci. 2019;9:1969.