The role of cannabinoids in CNS development: Focus on proliferation and cell death

Eduardo Cosendey Bockmann
Universidade Federal Fluminense https://orcid.org/0000-0002-0060-3667
Rafael Brito
Universidade Federal Fluminense https://orcid.org/0000-0002-7070-3348
Lucianne Fragel Madeira
Universidade Federal Fluminense https://orcid.org/0000-0001-6747-2828
Luzia da Silva Sampaio
Universidade Federal do Rio de Janeiro https://orcid.org/0000-0002-4944-7116
Ricardo Augusto de Melo Reis
Universidade Federal do Rio de Janeiro https://orcid.org/0000-0001-9689-4085
Guilherme Rapozeiro França (guilherme.franca@unirio.br)
Universidade Federal do Estado do Rio de Janeiro https://orcid.org/0000-0003-2324-4190
Karin da Costa Calaza
Universidade Federal Fluminense https://orcid.org/0000-0003-1199-1826

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Abstract

The active principles of *Cannabis sativa* are potential treatments for several diseases, such as pain, seizures and anorexia. With the increase in the use of *cannabis* for medicinal purposes, a more careful assessment of the possible impacts on embryonic development becomes necessary. Surveys indicate that approximately 3.9% of pregnant women use *cannabis* in a recreational and/or medicinal manner. However, although the literature has already described the presence of endocannabinoid system components since the early stages of CNS development, many of their physiological effects during this stage have not yet been established. Moreover, it is still uncertain how the endocannabinoid system can be altered in terms of cell proliferation and cell fate, neural migration, neural differentiation, synaptogenesis and particularly cell death. In relation to cell death in the CNS, knowledge about the effects of cannabinoids is scarce. Thus, the present work aims to review the role of the endocannabinoid system in different aspects of CNS development and discuss possible side effects or even opportunities for treating some conditions in the development of this tissue.

1. Introduction

Cannabinoid compounds were first identified in *Cannabis sativa*, which for centuries has been used as a medicinal herb. The most well-known and studied phytocannabinoid, which has a psychotropic effect, is delta-9-tetrahydrocannabinol (Δ^9-THC) (Pertwee 2006).

Classically, cannabinoid molecules act through two G protein-coupled receptors, CB1 and CB2 receptors, regulating several intracellular pathways. These receptors are mainly coupled to the inhibitory G protein, which, in turn, decreases the activation of adenylyl cyclase, reducing the formation of cAMP. In addition, they can block voltage-dependent Ca^{2+} channels and lead to the opening of K^+ channels, decreasing the release of neurotransmitters. These receptors are activated by endogenous molecules, known as endocannabinoids, working mainly as retrograde messengers (Harkany et al. 2007).

In addition to endogenous ligands and receptors, the endocannabinoid system is also composed of synthetic and degradation enzymes and membrane transporters that are present and functionally active since the early stages of CNS development. The endocannabinoidome is involved in cell fate, proliferation, migration, axonal growth and synaptogenesis, participating throughout embryonic development, intercellular communication and neural development (Alexandre et al. 2020; Garcia-Arencibia, Molina-Holgado, and Molina-Holgado 2019; Heimann et al. 2021; Leonelli et al. 2005). Therefore, changes due to exogenous substances might impact CNS development. Diverse studies have shown that early exposure to Δ^9-THC and/or to increased levels of endocannabinoids during development might lead to several behavioral changes, modification in the levels of transcription genes, and even cell death (Almada, Alves, et al. 2020; Campos et al. 2017; Philippot et al. 2019).

The use of phyto-, endo- or synthetic cannabinoids is becoming common in medicine due to the wide amount of data showing its beneficial effects for the treatment of several diseases (Baron 2018;

Levinsohn and Hill 2020; Sarris et al. 2020). Its therapeutic use is carried out through various mediators of the endocannabinoid system, including synthetic agonists and antagonists of both CB1 and CB2 receptors and enzymes such as FAAH (fatty acid acyl hydrolase) (Bonini et al. 2018). For example, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the two most abundant endocannabinoid compounds, have been shown to have analgesic and antiemetic effects, increase appetite and inhibit the growth of tumors. They have also been studied in the treatment of neurodegenerative diseases, epilepsy and psychiatric illnesses, such as anxiety and schizophrenia (Billakota, Devinsky, and Marsh 2019; Das et al. 2019; Sarris et al. 2020). Since some cannabinoids might be associated with anxiolytic effects as a “recreational drug”, caution should be taken. Therefore, based on scientific evidence, medicinal cannabinoid use is becoming more common in people's daily lives. However, the consequences of exposure during pregnancy need further attention.

According to the United Nations, more than 3.8% of people around the world use marijuana, and its use in pregnant women might be higher. It is estimated that 3.9% of women aged 18–44 years use *cannabis* during the last month of pregnancy. Moreover, its use increases to 7.5% when the evaluation is restricted to a younger range of pregnant people, 18–25 years (Corsi et al. 2019). One study showed that the number can be even higher, as 19% of women aged 18–24 years have used *cannabis* during pregnancy (Young-Wolff et al. 2017).

Increased use of *cannabis* is also linked to greater approval for medicinal use, particularly for the treatment of Lennox-Gastaut syndrome, a severe childhood epileptic encephalopathy, and for the relief of symptoms of multiple sclerosis (Huestis et al. 2019). Its medicinal use is mainly due to the manipulation of specific *cannabis* compounds. Currently, an oromucosal spray composed of THC and cannabidiol (CBD) is approved (in European and non-European countries) with the aim of alleviating the symptoms of multiple sclerosis. In this case, CBD and THC correspond to at least 90% of the extract, but they have other constituents that can contribute to the effects. However, drugs related to the cannabinoid system can cause side effects, especially due to THC and its psychoactive effect, limiting the dose that can be used. In the case of administration by spray, even in one single dose, the compounds are rapidly absorbed, and the increase in the number of doses of the spray leads to a proportional increase in its escalation of the pharmacological response (Rog, Nurmikko, and Young 2007). The use of this drug does not lead to its accumulation when used at multiple doses; thus, it may have limited side effects. Therefore, it is indicated that the THC/CBD spray is well tolerated and has few side effects, with no sequelae, although there are side effects such as headache, drowsiness, loss of attention, euphoria and dizziness (Stott et al. 2013).

The use of highly purified CBD treatment has been approved in the US for the treatment of Lennox-Gastaut syndrome, primarily for its anticonvulsant effects. It was shown that a single dose of CBD is well tolerated with very few adverse effects and has no drug accumulation (Tayo et al. 2020). Corroborating these data, another study evaluated the effects of CBD and its adverse effects, despite being well tolerated; the use of CBD might cause diarrhea, nausea, headache and drowsiness as the main side effects. In addition to being well tolerated in a single dose, daily doses over 3 months were well tolerated

in the treatment of epilepsy and multiple sclerosis (Taylor et al. 2018). There is great potential for the use of cannabinoids, especially CBD, in psychiatric illness due to their anxiolytic, antidepressant and antipsychotic effects. However, little is known about the effects on development and what its use during pregnancy might cause.

As previously mentioned, cannabinoids play a role during embryonic development. Therefore, interference in the endocannabinoid system during development might cause complications, such as intrauterine growth restriction, miscarriages, and risk of neonatal morbidity (Campos et al. 2017; Fernández-ruiz et al. 2000; Galve-Roperh et al. 2006; Harkany et al. 2008; Maia et al. 2020; Prenderville, Kelly, and Downer 2015). Some of these effects from cannabinoids during pregnancy may be due to alterations in and through the placenta, as Δ^9-THC is able to cross the placental barrier, and *cannabis* appears to increase placental permeability to pharmacological and recreational substances, representing another potential risk to the fetus. Exposure to cannabinoids could modify developmental events, such as proliferation, migration, differentiation and survival, in addition to changing the maturation of neurotransmitters (Navarrete et al. 2020).

Nevertheless, it is still complex to verify the possible effects of cannabinoids during pregnancy and in fetal development, since it is difficult to exclude exposure to other commonly used substances, such as tobacco and alcohol, among others, interfering with the evaluation of the possible isolated actions of cannabinoids. In addition, major concerns due to the effects of cannabinoids in conjunction with other drugs are appealing, since it is common to use them together. For example, supraphysiological exposure of exogenous cannabinoids to the fetus may amplify apoptosis caused by ethanol in the rat brain (Hansen et al. 2008).

As mentioned above, the cannabinoid field has dedicated much effort to study and establish the role of the endocannabinoid system in the physiology and pathology of the mature CNS. However, the early effects of cannabinoids in CNS development have been much less explored. Therefore, the present review aims to provide information regarding the role of the endocannabinoidome during early CNS development, especially on proliferation and cell death. Considering a field still in its infancy to be much explored, the collection of data in the present work raises the possibility of a caution effect of cannabinoid exposure during CNS development.

2. Search Strategy And Selection Criteria

A detailed search in Medline database was conducted for all published reviews and research data. In the present review the inclusion criteria were scientific articles or reviews mainly from 2015 to 2021, with CNS or proliferation, differentiation, cell fate, cell death, (endo) cannabinoids in the title and abstract. Although older data and reviews were included due to the unique character and little information on some subjects. Articles from both *in vitro* and *in vivo* studies were included. For *in vivo* studies we selected only those that used vertebrate animals, to have a closer comparison with humans.

3. Endocannabinoid System

3.1. ENDOCANABINOIDS:

The endocannabinoid system is a physiological system that controls neural functions as a circuit breaker, is related to inflammatory and vascular processes and is highly conserved among animal species, including humans. It is composed of two main G protein-coupled receptors, CB1 and CB2, and several endogenous ligands, the most common of which are N-arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG), in addition to enzymes involved in their synthesis and degradation (Garcia-Arencibia, Molina-Holgado, and Molina-Holgado 2019).

Cannabinoid research began by the search for substances responsible for marijuana effects. After the isolation of THC from *cannabis*, radiolabeled THC allowed the purification of CB1 and CB2 (Pacher, Kogan, and Mechoulam 2020). The discovery of the CB1 receptor led to a search for possible endogenous ligands. AEA was discovered from purified fractions from porcine brain, which contained a molecule able to inhibit the binding of an exogenous agonist (HU-243) of CB1 and CB2 receptors (Hanuš 2007). AEA was the first endocannabinoid discovered and is the most described in the literature so far (Hanuš 2007), and together with 2-AG, it represents the most prevalent endogenous lipid ligand of the cannabinoid system, known as endocannabinoids.

AEA and 2-AG are lipophilic and are not stored in synaptic vesicles as are most neurotransmitters. They are produced on demand by the action of calcium-dependent phospholipases and function as retrograde messengers (Fig. 1). AEA is part of the family of bioactive lipids, N-acylethanolamines (NAEs), sharing in common their metabolic pathways for neural generation and inactivation (Nyilas et al. 2008). AEA synthesis occurs mainly through the increase in intracellular Ca^{2+}, inducing the action of the enzyme phospholipase-D (NAPE-PLD), which hydrolyzes N-arachidonoyl phosphatidylethanolamine (NAPE) (Fig. 1). However, there are also other possible ways for AEA synthesis: the phospholipase C (PLC), secreted phospholipase A2 (sPLA2), ABHD4 and glycerophosphodiester phosphodiesterase 1 (GDE1) pathways (Ueda, Tsuboi, and Uyama 2013). The synthesis of 2-AG is stimulated by high levels of Ca^{2+} and the Gq/phospholipase C pathway, which produces diacylglycerol (DAG). In addition, phosphatidic acid (PA) and phosphoslipase A1 (PLA_1) also produce 2-AG, as PA is hydrolyzed by PA phosphohydrolase (PAP), producing DAG, while PLA_1 converts phosphatidyl lipids into 2-arachidonoyl-lyso PI, which is further converted into 2-AG (Murataeva, Straiker, and MacKie 2014). Thus, the isoenzymes diacylglycerol lipase (DAGL), DAGLα and DAGLβ hydrolyze DAG, ultimately generating 2-AG (Fig. 2) (Murataeva, Straiker, and MacKie 2014; Ueda, Tsuboi, and Uyama 2013).

Endocannabinoids are degraded by specific enzymes. Fatty acid amide hydrolase (FAAH) is primarily a postsynaptic integral membrane protein that degrades AEA into arachidonic acid (AA) and ethanolamine, and it is the main catabolic enzyme for the fatty acid amide lipid (FAA) class (Fig. 2). Later, the FAAH-2 enzyme was also discovered in humans, and it has the ability to hydrolyze AEA and palmitoylethanolamide (PEA), contributing to the degradation of NAEs (Kaczocha et al. 2010; Wei et al.

2006). AEA can also be degraded by N-acylethanolamine amidase (NAAA) in AA and ethanolamide (Macarrone et al 2015). Knockout (KO) mice for FAAH-/- exhibit increased endogenous cannabinoid activity and a reduced perception of pain (Cravatt et al. 2001). These effects are probably due to high levels of AEA and other NAEs, such as oleoylethanolamide (OEA) and PEA, which were 15 times higher (pmol/g) in comparison to the wild type (Cravatt et al. 2001). It has also been shown that pharmacological inhibition of FAAH increases AEA levels, which regulates nociception, confirming the analgesic effect observed in KO $FAAH^{-/-}$ animals (O'Sullivan 2016).

Monoacylglycerol lipase (MAGL), an endocannabinoid hydrolytic enzyme, is primarily a presynaptic cytosolic enzyme that degrades 2-AG in AA and glycerol (Fig. 1). In the brain, most (~ 85%) degradation of 2-AG is carried out by MAGL. However, there is also a smaller participation of the α/β-hydrolase domain-containing 6 (ABHD6) and α/β-hydrolase domain containing 12 (ABHD12) enzymes (Fig. 2) (Saario et al. 2005). These main hydrolases act in different subcellular compartments, in which ABHD12 has an extracellular orientation, while ABHD6 has a cytoplasmic and integral membrane orientation (Fig. 1). However, MAGL is localized in the cytoplasm and the soluble/peripheral membrane. This distinction in the location of hydrolase enzymes possibly explains the difference in the participation of 2-AG degradation, as ABHD6 and ABHD12 are responsible for ~ 4% and ~ 9%, respectively (Blankman, Simon, and Cravatt 2009; Gulyas et al. 2004). Muccioli and coworkers (2007) described another MAGL in the mouse microglial cell line BV-2. The authors verified that although this cell line did not express the original MAGL mRNA, the hydrolysis of 2-AG still occurred (Muccioli et al. 2007). Finally, FAAH can also hydrolyze 2-AG to a lesser extent into AA and glycerol (Murataeva, Straiker, and MacKie 2014).

There is also the occasional participation of cyclooxygenase-2 (COX-2), lipoxygenase-12 and − 15 (LOX-12/-15) and cytochrome p450 oxygenase that metabolize both AEA and 2-AG (Fig. 2) (Parker 2017; Pinho Costa et al. 2011; Redmond et al. 2014).

Primary metabolic pathways of AEA and 2-AG synthesis and degradation. AEA synthesis – An increase in Ca^{2+} stimulates NAPE-PLD to hydrolyze NAPE, producing AEA. PLC catalyzes the formation of phospho-NAE, and the phosphatases PTPN22 and INPP5D then convert phospho-NAE to AEA. NAPE is catalyzed by sPLA2 or ABHD4, generating lyso-NAPE. Then, ABHD4 catalyzes the formation of GP-NAE, which is converted to AEA by the phosphodiesterase GDE1. 2-AG synthesis – PLC mediates the hydrolysis of PIP_2, which produces DAG, which is converted to 2-AG by DAGLα/β. Phosphatidic acid (PA) is hydrolyzed by PA phosphohydrolase, producing DAG, which is converted by DAGLα/β into 2-AG. AEA degradation – AEA is degraded primarily by FAAH into AA and ethanolamine, while COX-2 degrades into prostamide. 2-AG degradation – 2-AG is degraded primarily by MAGL into AA and glycerol. ABHD6 and ABHD12 also degrade 2-AG into AA and glycerol, although they are less common. COX-2 degrades 2-AG into PGE2-G.

Beyond the classic lipid endocannabinoids, a new class of peptides known as hemopressins act as modulators of cannabinoid receptors, especially as inverse agonists of CB1R (Heimann et al. 2021). RVD hemopressin, an N-terminally extended form of hemopressin, acts as a negative allosteric modulator of CB1R; however, it is a positive allosteric modulator of CB2R. In addition to modulating classical

cannabinoid receptors, it has been described that hemopressins can modulate other receptors and components of the endocannabinoid system (Riquelme-Sandoval et al. 2020).

Endocannabinoid components are present since the early stages of CNS development, and exposure to substances that change endocannabinoid availability or cannabinoid receptor activity may impact CNS development. On the other hand, with an increase in the comprehension of cannabinoid function during CNS development, strategic cannabinoid interventions for pathological conditions may also arise.

3.2. *CANNABIS*:

Cannabis sativa has been used for over 5000 years in a medicinal and recreational way (Pertwee 2006). The use of *cannabis* as a medicinal herb was first reported in China, approximately 2727 B.C. Since then, *cannabis* has been used as a medicinal agent with antinociception, anti-inflammatory, anticonvulsant, and antiemetic effects, in addition to its recreational use. Over 100 different types of phytocannabinoids have been isolated from the plant. Cannabinol was the first phytocannabinoid discovered and isolated (Pertwee 2006). The main components of the plant are cannabidiol (CBD), with no psychoactive properties, and Δ^9-THC, which is the main psychoactive component of the plant (Parker 2017). There are several other constituents much less studied, such as cannabigerol (CBG), cannabichromene (CBC), cannabidivarin (CBDV), and tetrahydrocannabivarin, among others (Russo 2019). In addition to phytocannabinoids, there are several other phytomolecules in *cannabis*, such as terpenes and flavonoids. Under several circumstances, the combination of these compounds leads to an improved effect compared to an isolated molecule, thus denominated the entourage effect (Koltai and Namdar 2020), also described for endocannabinoids. The biological activity of 2-AG, for example, is increased when 2-linoleoyl-glycerol and 2-palmitoyl-glycerol are simultaneously administered. The latter substances usually do not bind to cannabinoid receptors and neither inhibit the activity of adenylyl cyclase (AC), a classical mechanism of action of 2-AG in Gi protein-coupled receptors CB1 and CB2. However, when combined with 2-AG, they enhance the inhibition of AC. In addition, together, they also increase the effect of 2-AG on the inhibition of motor behavior, immobility on a ring, analgesia on a hot plate and hypothermia in mice (Pacher, Kogan, and Mechoulam 2020). An entourage effect is also observed when AEA and palmitoylethanolamide (PEA) are coinjected intraperitoneally in rats, causing hypotensive effects mediated by TRPV1 and CB1 activation, while AEA alone is ineffective (García, Adler-Graschinsky, and Celuch 2009). Therefore, PEA improves the effects mediated by AEA and capsaicin on Ca^{2+} influx, mainly due to an increase in the modulation of TRPV1 activity caused by the entourage effect (Pacher, Kogan, and Mechoulam 2020).

Although not confirmed, it is possible that the whole plant extracts may be more effective for medicinal treatment than an isolated compound. Therefore, it may be possible in the future to take advantage of the entourage effect in particular pathological conditions of the CNS in developmental stages.

3.3. CANNABINOID RECEPTORS

Classically, cannabinoids can act via two presynaptic metabotropic receptors, CB1 and CB2. CB1 receptors are considered to be the most abundant metabotropic receptors in the CNS of mammals, in addition to being also present in peripheral tissues. They are mainly expressed in presynaptic terminals, regulating the release of several neurotransmitters, such as GABA, glutamate, serotonin, acetylcholine and dopamine (López-Moreno et al. 2008).

CB1 receptors are found in higher concentrations in neocortex, hippocampus, basal ganglia, cerebellum and brain stem, in addition to other parts of the CNS, such as the retina (Mackie 2005; da Silva Sampaio et al. 2018). CB1R is present at high levels in presynaptic terminals. CB1R is also present in postsynaptic terminals of neurons, astrocytes and oligodendrocytes (Busquets-Garcia, Bains, and Marsicano 2018; Han et al. 2012). It regulates molecular and synaptic functions controlling, consequently, different behavioral responses, such as food intake, stress and memory processes (Busquets-Garcia, Bains, and Marsicano 2018; Kendall and Yudowski 2017). Like many other GPCRs, the CB1 receptor is primarily located on the surface of cells, the plasma membrane, where it performs its most well-known functions, inhibition of adenylyl cyclase with the consequent reduction of cAMP levels. CB1 receptors can also block voltage-dependent calcium channels, decreasing exocytosis and neurotransmitter release. However, CB1 might also be found in other subcellular compartments (Zou and Kumar 2018). In the lysosome, the CB1 receptor increases the cytoplasmic calcium concentration through the release of internal calcium stores in addition to the lysosomal calcium per se due to the increase in lysosomal permeability (Zou and Kumar 2018). In the mitochondria, it acts directly to inhibit cellular respiration and the production of cAMP, regulating energy metabolism (Bénard et al. 2012). Finally, CB1R in the endosome can regulate β-arrestin signaling pathways (Kendall and Yudowski 2017; Zou and Kumar 2018). The above data reveal the relevance of CB1 receptors in the functionality of the CNS. Meanwhile, they are also related to different kinds of CNS disorders, such as Huntington disease, multiple sclerosis, Alzheimer's disease, anorexia, posttraumatic stress, schizophrenia, and eating and alcohol use disorders (Cristino, Bisogno, and Di Marzo 2020; Kendall and Yudowski 2017).

CB1 receptors also activate several intracellular pathways, such as the signaling pathways of the three subfamilies of the mitogen-activated protein kinase MAPK, the kinase 1/2 regulated by extracellular signal (ERK1/2), the c-Jun-N-terminal (JNK) kinase and p38, which are involved in the regulation of main development processes such as cell proliferation, control of cell cycle and cell death. In addition, it has been observed that the CB1 receptor can exert its protective effects through the activation of the PI3K/AKT pathway, which is important for cell growth and controlling cell death (Galve-Roperh et al. 2002; Gómez Del Pulgar, Velasco, and Guzmán 2000; Zou and Kumar 2018).

Classically, the CB2 receptor is described as mainly found in the immune system. However, it has also been described in the mature CNS, even though it is less abundant than CB1. Although the exact functions of CB2R in the CNS remain to be revealed, it has been frequently associated with inflammatory processes (Kendall and Yudowski 2017; Mccoy 2016). CB2R is present in microglia, astrocytes and neurons, and it is involved in synaptic function control and plasticity (Campos et al. 2017; Freund, Katona, and Piomelli 2003; Kendall and Yudowski 2017). It is present in the prefrontal cortical pyramidal neurons,

where it modulates neuronal excitability through the regulation of Ca^{2+}-activated Cl^- channels (Zou and Kumar 2018). It has also been described that CB2R activation inhibits acute nociception, inflammatory hyperalgesia, and neuropathic sensory hypersensitivity (Ibrahim et al. 2006). CB2R is also related to neuropsychiatric disorders, including alcoholism, eating disorders, depression, schizophrenia, and autism spectrum disorders (Chen et al. 2017; Cortez et al. 2020).

Cannabinoids can also bind to other receptors, such as the family of nuclear receptors activated by peroxisome proliferators (PPARs), also targeted by their derivatives PEA and OEA (O'Sullivan 2016). Cannabinoids might also activate the G55 protein-coupled receptor (GPR55), considered an orphan receptor, and GPR18, which has been associated with the endocannabinoid system, although it is not known whether CB1 or CB2 receptor ligands can activate or block GPR18 (Morales and Reggio 2017). There are discussions about GPR55 being considered a third type of cannabinoid receptor (CB3); however, this nomenclature is still controversial because there are other ligands for GPR55 (Sharir and Abood 2010). Finally, cannabinoids can still regulate some transient receptor potential (TRP) channels and ionotropic receptor members (Muller, Morales, and Reggio 2019). It has been identified that vanilloid TRP (TRPV1, TRPV2. TRPV3, TRPV4), anquirin TRP (TRPA1) and melastatin TRP (TRPM8) can be modulated by cannabinoids (Muller, Morales, and Reggio 2019).

In addition to the classical action mechanisms, studies have shown that G protein-coupled receptors, such as CB1 and CB2 receptors, can form both homodimers and heterodimers. Both heterodimers of CB1 and CB2 receptors have been studied. CB1 receptors can form heterodimers with receptors for serotonin, angiotensin, opioids, somatostatin, dopamine, and GPR55, among others (Martínez-Pinilla et al. 2014; Turu and Hunyady 2010; Viñals et al. 2015; Zou, Somvanshi, and Kumar 2017). On the other hand, the formation of heterodimers by CB2 may be associated with an interaction with CB1, GPR55, serotonin receptor 5HT1A or the chemokine receptor CXCR4 (Coke et al. 2016). This oligomerization can affect receptor signaling, cannabinoid receptor trafficking and ligand binding. Thus, some "nonclassical" responses associated with cannabinoid receptor stimulation could be explained by this oligomerization. An example is the effect of intracellular calcium mobilization by a wide range of CB1 agonists in the neuroblastoma glioma cell line NG108-15 (Sugiura et al. 1996). In addition, an interaction of some of these heteromers is also related to the progression of cancer and neurodegenerative diseases (Coke et al. 2016). However, many studies are still necessary to expand knowledge regarding this mechanism (Mackie 2005; Morales and Reggio 2017).

Endogenous substances, such as AEA and 2-AG, mainly act on CB1 and CB2 receptors. AEA can be considered a partial agonist of CB1 and CB2 receptors; it is less effective than 2-AG and Δ^9-THC and has a lower affinity for CB2 than CB1. AEA can also target TRPV1 and TRPA1 channels, PPAR-α and γ, GPR55 and GPR119 (Maccarrone 2017; Muller, Morales, and Reggio 2019; Reggio 2010; Solymosi and Kofalvi 2016). 2-AG acts as a full agonist for the CB1 and CB2 receptors and can also bind to PPAR-α and γ, TRPV1 and GPR55 (Baggelaar, Maccarrone, and van der Stelt 2018; Campos et al. 2017). However, the phytocannabinoid Δ^9-THC mimics endocannabinoids as a partial agonist at CB1 and CB2 receptors (Pacher, Kogan, and Mechoulam 2020). On the other hand, CBD acts as a very weak inverse agonist of

the CB1 and CB2 receptors and as an agonist of the TRPV1 receptor. PEA and OEA lack affinity at CB1/CB2R but evoke pharmacological effects and may act indirectly on them (Borrelli et al. 2015; Brown 2007). Moreover, there are synthetic ligands, such as WIN55212-2, which is a nonselective agonist of both the CB1 and CB2 receptors, while AM-694 is a potent and selective agonist of CB, and AM-1221 is a selective agonist for the CB2 receptor (Guzmán 2003; Peres et al. 2018; Schwitzer et al. 2016). Among the most commonly used inhibitors are SR141716A, AM251, AM281 and LY320135, antagonists selective for the CB1 receptor, and SR144528 and AM630 for the CB2 receptor, which behave as inverse agonists (Pertwee 2010).

There are other nonendocannabinoid compounds that can also bind to nonclassical cannabinoid receptors, such as PEA and OEA, which are part of N-acylethanolamine. PEA is a component found in foods with anti-inflammatory, analgesic, anti-epileptic and neuroprotective effects by acting through PPAR (O'Sullivan 2016). OEA comes from dietary fat digested in small intestine enterocytes and causes satiety and reduces body weight gain. OEA signaling dysfunction can contribute to overweight and obesity (Tsuboi et al. 2018).

4. Cannabinoid In Early Development: Pregancy

The vast amount of information about the medicinal properties of *cannabis* impacts legalization and increases medicinal usage in several countries around the world (Bonini et al. 2018; Levinsohn and Hill 2020). However, for a specific group of people, especially during pregnancy, the consumption could be hazardous. A study in the United States found that approximately 7.5% of pregnant people between the ages of 18–25 years use *cannabis* (Grant et al. 2018). Specifically, in California, a study found that approximately 7% of pregnant women surveyed used *cannabis*, and an impressively 19% of women aged 18–24 years used *cannabis* in this period (Young-Wolff et al. 2017). *Cannabis* usage during pregnancy is associated with restricted intrauterine growth, premature labor and fetal neurodevelopmental disorders (Grant et al. 2018). Exposure to cannabinoids throughout the gestational period and/or lactation may result in developmental changes in fetuses and neonates.

AEA is present since the origin of blastocysts and in the early embryonic stages and is important for the maintenance of pregnancy. Increased AEA levels impair embryo development and implantation, while endocannabinoids at lower levels stimulate growth and differentiation via CB1 (Paria et al. 2001). It has been shown that pregnant rats administered anandamide (3 mg/kg) from gestational day 7 to postnatal day 21 exhibit a higher number of stillborn and pups with low body weight than control rats. In the case of females treated with AEA, there is an increase in the expression of CB1 receptors in the brain but not in the offspring (Amlani et al. 2017). Interestingly, the placenta of people who have suffered miscarriage has high expression of the CB1 receptor and low expression of FAAH (Trabucco et al. 2009), which raises the hypothesis that hyperactivity of the cannabinoid system in placental tissue is involved in dysfunctions of the placenta with consequent abortion. Accordingly, AEA can induce cell death in cultured rat decidual cells. During pregnancy, the uterus undergoes morphological and physiological changes, in addition to apoptosis and necrosis, which lead to regression of the decidual tissue. In this case, it was

found that AEA induced cell death by apoptosis in a dose- and time-dependent manner. AM251, a CB1 receptor antagonist, blocked cell death, while the CB2 and TRPV1 antagonists AM630 and capsazepine, respectively, had no effect. The work also found that pretreatment with methyl-β-cyclodextrin (MβCD), a cholesterol depletory, increased cell viability and decreased LDH release. Thus, AEA can induce cell death in deciduous cells mediated by the CB1 receptor with the involvement of cholesterol-rich lipid rafts (Fig. 3) (Fonseca, Correia-da-Silva, and Teixeira 2009). In a later study, it was found in rats that 2-AG may also be related to decidual cell death. The levels of 2-AG are higher than those of AEA in the uterus and brain (Wang et al. 2007). 2-AG (25 μM) induced deciduous cell death mediated by the CB1 receptor, while at 50 μM, it caused a more intense change in cell morphology and viability. The cytotoxic effect of 2-AG at a higher concentration was inhibited by MβCD. It is possible that the cell death caused by both AEA and 2-AG is related to COX-2, since it has been demonstrated that AEA-derived oxidative metabolites induce cell death in several cell lines, and the oxygenation of 2-AG by COX-2 may be related to some of these effects (Fig. 3) (Fonseca et al. 2010). 2-AG can also cause reticulum stress and cell death in the placenta via CB2 receptor signaling. This activation leads to endoplasmic reticulum stress-induced apoptotic cell death through PERK-ATF4-CHOP. Furthermore, this stress is also probably related to the increase in ROS production induced by 2-AG (Fig. 3) (Almada, Costa, et al. 2020). According to this idea, BeWo cells, a human cytotrophoblastic cell lineage, undergo apoptosis when exposed to THC or synthetic cannabinoids (JWH-018, JWH-122, UR-144) by a mechanism that depends on increased ROS and endoplasmic reticulum stress (Fig. 3) (Lojpur et al. 2019; Maia et al. 2020). Cannabinoids were also described in human granulosa cells (hGCs) and the COV434 granulosa cell line. AEA treatment in hGCs and in the COV434 cell line decreased cell viability and induced apoptosis, causing an increase in caspase 2/7 activities, and in hGCs, it also increased caspase 8 activity (Costa et al. 2021).

It has been previously shown that there is also a correlation between the levels of uterine AEA and the status of autophagy in blastocysts. Autophagy activation and AEA levels are higher at the beginning of pregnancy in mice; however, they are intensely decreased near the time of implantation (Lee et al. 2011). When the synthetic agonist methanandamide (at 28 nM) was administered to pregnant mice, it enhanced the autophagy process in preimplanted mouse embryos, prolonging autophagy activation and causing cell death by apoptosis. Therefore, although AEA and autophagy are important for embryo implantation, increased levels of this endocannabinoid lead to changes in development and survival, extending the activation of autophagy that can lead to cell death (Oh et al. 2013).

Thus, the increase in stillborn may be related to a possible increase in the expression and activity of CB1 receptors in the placenta of pregnant women treated with AEA, which causes placental dysfunction by increasing the death of trophoblastic cells. However, more work needs to be done in this area to better elucidate this point. Therefore, identifying the roles of endocannabinoids in neurodevelopment is crucial to evaluate the possible side effects of the modulation of the cannabinoid system.

Apoptosis induced by cannabinoids. AEA and 2-AG activate CB1R and COX-2, inducing apoptosis in a manner dependent on the cholesterol-rich lipid rafts in cultured decidual cells. 2-AG causes an increase in ROS, leading to reticulum stress and apoptosis through PERK-ATF4-CHOP in the placenta via CB2R. 2-AG,

Δ9-THC and synthetic cannabinoids increased ROS, causing reticulum stress-induced apoptosis in the cytotrophoblastic cell lineage.

4.1.1. ENDOCANNABINOID SYSTEM IN THE CNS DEVELOPMENT:

The endocannabinoid system is present and functional from the early stages of nervous system development and has several specific roles. The bioavailability of endocannabinoids 2-AG and AEA varies throughout brain development. Analysis by gas chromatography/mass spectrometry showed that AEA concentration levels are limited in the brains of rats in midgestation, increasing from the perinatal period to the adult period (Harkany et al. 2008). During neural tissue differentiation, endocannabinoids may function as autocrine mediators instead of classic retrograde signaling (Keimpema et al. 2010). 2-AG production increases during tissue differentiation, including in the CNS (Bisogno et al. 2003; Keimpema et al. 2010). Moreover, the expression of DAGLα and DAGLβ enzymes is increased in axonal tracts during the middle and final gestational periods of mice, exceeding in comparison to later periods of development (postnatal), being responsible for the delay in 2-AG appearance (MacCarrone et al. 2014). Accordingly, DAGL is important for axonal growth (Brittis et al. 1996). DAGLα and DAGLβ are expressed in axons at embryonic day 10 (E10) in mice, crossing the floor plate of the spinal cord, and at E14, DAGLα and DAGLβ are present in the retinal nerve fiber layer and in the optic nerve (Bisogno et al. 2003). They can also be seen in the cerebellum, dendritic field and deep cerebellar nuclei (Bisogno et al. 2003).

FAAH is also present during brain development. It has been observed in radial glia of the hippocampus during late gestation and postnatal periods *in vitro* and *in vivo* (Aguado et al. 2006). Neural progenitors express FAAH at postnatal day 2 in mice. FAAH is also expressed in undifferentiated neural cells, participating in astrogliogenesis and neural progenitor differentiation *in vivo* (Basavarajappa, Nixon, and Arancio 2009). FAAH expressed in hippocampal progenitor cells may be involved in hippocampal development (Aguado et al. 2006). FAAH KO adult mice showed enhanced hippocampal proliferation and astroglial differentiation (Aguado et al. 2006). MAGL distribution, expression and function during the developing nervous system is still unclear. As shown, MAGL distribution changes during synaptogenesis. An *in vitro* study showed that MAGL accumulates in growth cones that oppose a postsynaptic neuron but not in growth cones of collateral axons that have not selected a target (Keimpema et al. 2010). Moreover, MAGL changes the axonal growth rate, modulates direction and determines the size of axons (Keimpema et al. 2010). However, when MAGL is inhibited, it can trigger neurite outgrowth in neurons in both cells that emit a tunted quiescent axon shortly after plating and neurons with an established primary neurite. After synaptic wiring, DAGL and MAGL are redistributed in neurons, with DAGL becoming exclusively present in somatodendritic domains of neurons and MAGL becoming located in the presynaptic neuronal site (Basavarajappa, Nixon, and Arancio 2009; Keimpema et al. 2010; MacCarrone et al. 2014). In the mature synapse, it is possible to verify the participation of ABHD6 and ABHD12 in addition to the degradation enzymes already mentioned (Bari et al. 2005; MacCarrone et al. 2014).

CB1 and CB2 are the main receptors of the endocannabinoid system involved in CNS development. CB1 and CB2 are expressed by embryonic stem cells and neural progenitor cells, which regulate proliferation and cell fate. They usually show opposite patterns of expression in neuronal progenitors, with CB1 increasing while CB2 decreasing during neuronal differentiation (Galve-Roperh et al. 2013). CB1 and CB2 are also detected in oligodendrocyte progenitors (Molina-Holgado et al. 2002). CB2 receptors are present in neural progenitors during embryonic stages until adulthood. The CB1 receptor is expressed at high levels in differentiated neurons and at lower levels in glial cells in the hippocampus, basal ganglia and cortex. The presence of CB1 receptor mRNA transcripts was also seen in the forebrain, subventricular zones of the striatum, nucleus accumbens and neocortex (Fernández-ruiz et al. 2000). CB2 during development is present in microglial and macrophage cells (Zurolo et al. 2010).

4.1.2. EARLY STAGES OF CNS DEVELOPMENT: CELL FATE AND PROLIFERATION

The endocannabinoid system appears to be involved in all stages of CNS development since neurulation. Previous work on chicken embryos analyzed the influence of cannabinoids in the early stages of development. Exposure to Δ^9-THC during developmental stages 3–8, which corresponds to 2–3 gestational weeks in humans, before neurogenesis and somitogenesis causes deregulation of intrinsic factors important for CNS development. For example, the Sox2 expression domain appeared to be reduced in treated embryos, while Pax6 was downregulated in nascent neural tubes. Moreover, Δ^9-THC causes abnormal formation of neural plaques, interfering with the initial stages of the CNS (Psychoyos et al. 2008; De Salas-Quiroga et al. 2015).

Cell fate and proliferation of neural progenitors are also influenced by cannabinoids. In two human induced pluripotent stem cell (hiPSC) lines, Δ^9-THC, as well as two synthetic CB agonists (THJ-018 and EG-18), decreased the PAX6 neural progenitor marker while increasing the Hu/C marker of early differentiated neurons (Miranda et al. 2020). In addition, an increase in GFAP-positive cells and a decrease in the synaptophysin marker were shown, suggesting that these cannabinoid agonists anticipate progenitor commitment, promoting glial cell fate (Miranda et al. 2020). Finally, it is still uncertain all the physiological processes that may be altered by *cannabis* during development. Activation of the CB1 receptor on neural stem cells derived from embryos increases the differentiation of progenitor cells into neurons. The CB1 receptor coordinates the intrinsic neurodevelopment program during neuronal development. In R1 mouse embryonic stem cells in culture, cell fate default in deep layer pyramidal neurons is accompanied by increased expression of cannabinoid system components, such as CB1, DAGL, NAPE-PLD, FAAH, 2-AG and AEA (Paraíso-Luna et al. 2020). R1 cells that develop in the presence of an MAGL inhibitor (JZL-184, 1 μM) or in the presence of CB1 agonists HU-210 (100 nM) or Δ^9-THC (2 μM) have a greater number of deep layer pyramidal neurons than upper layer pyramidal neurons, an effect that depends on the ERK and AKT pathways. Interestingly, brain organoids exposed to CB1 agonists have a higher density of pyramidal neurons in the deep layer than neurons in the upper layers (Paraíso-Luna et al. 2020). Together, these data demonstrate that CB1 signaling is involved in the development and

maturation of deep layer pyramidal neurons. Endocannabinoids can also inhibit neuronal differentiation *in vitro* in cortical neuron progenitors obtained from E17 old embryos. They influence cell fate by interfering with the ERK signaling pathway. Endocannabinoids inhibit NGF-induced signaling events, resulting in the inhibition of neuronal generation (Rueda et al. 2002).

Several data in the literature correlate the endocannabinoid system with the proliferation of neural progenitors in different experimental paradigms. In the olfactory tissue, intranasal instillation of 2-AG (1–10 μM) or WIN 55,212-2 (10 μM) increases the proliferation of neural progenitors in the olfactory epithelium of neonate mice (0–4 days postnatal), as measured by the incorporation of BrdU (Hutch and Hegg 2016). Treatment with eicosapentanoic acid in neural stem cells increases 2-AG levels, which activates the p38 MAPK signaling pathway through CB1 and CB2, increasing cell proliferation (Dyall et al. 2016). Accordingly, neuronal progenitors from CB1 KO showed a decrease in cell renewal and proliferation (Campos et al. 2017). There is also evidence that CB1 receptors may participate in cell proliferation in the subventricular zone. In fact, activation of the CB1 receptor by the selective agonist ACEA (1 μM), (R)-(+)-methanandamide (Rm-AEA, 1 μM) or HU-210 (5 μM) induces cell proliferation in subventricular neurospheres derived from postnatal (P1-3) rats (Rodrigues et al. 2017; Trazzi et al. 2010; Xapelli et al. 2013). The proliferative response triggered by the CB1 receptor is inhibited by treatment with AM251 (CB1 antagonist), AM630 (CB2 antagonist) (Rodrigues et al. 2017), the BDNF scavenger (Ferreira et al. 2018) and PI3K/AKT inhibitors (Trazzi et al. 2010). Although CB1 is more abundant in the brain, CB2 also plays a role in proliferation. Interestingly, the combined administration of ACEA and HU-308 does not alter cell proliferation in neurospheres, which is promoted by a CB2 antagonist. Together, these findings suggest that the balance between CB1 and CB2 receptor activation orchestrates cell proliferation in neurospheres of the subventricular zone. In addition, BDNF signaling and the PI3K/AKT pathway are closely involved in this response (Ferreira et al. 2018; Rodrigues et al. 2017; Trazzi et al. 2010). The PI3K/AKT pathway is also important for the proliferation of granular cerebellar precursor cells. In fact, the increased proliferation of these cells in culture by HU-210 is dependent on the phosphorylation of AKT and GSK3β and translocation of β-catenin to the nucleus, where it increases the expression of cyclin D1 mRNA (Trazzi et al. 2010). Moreover, activation of CB1 in the hippocampus modulates the fate of neural progenitor cells, promoting proliferation, in a similar way observed in SVZ neurospheres. CB1 also inhibits differentiation, and all these effects are inhibited by the CB1 antagonist SR141716 (Jiang et al. 2005). In SVZ and DG neurospheres, simultaneous activation of CB1 and CB2 led to an increase in neuronal proliferation at early stages of development in Sprague-Dawley rats (Alexandre et al. 2020; Rodrigues et al. 2017), showing that the outcome of concomitant activation of CB1 and CB2 receptors may depend on the brain area. In addition, blocking the CB2 receptor with AM630 (1 μM) also inhibits BDNF-induced cell proliferation in neurospheres derived from the dentate gyrus and subventricular zone of postnatal (P1-3) mice (Ferreira et al. 2018). In the lineage of striatum neural progenitors ST14A, the 24-hour administration of 2-AG (5 μM) or CB2 selective agonist (JHW133, 300 nM) increases cell proliferation in culture, which is blocked by the selective CB2 inhibitor (AM630, 300 nM) as well as U73122 and wortmannin, PLC and PI3K blockers, respectively (Cottone et al. 2021). Although the ST14A lineage expresses CB1, they do not appear to participate in cell proliferation in this case. Thus, cell proliferation of striatal progenitors in

culture depends on the activation of the CB2 receptor and on the activation of the PLC and PI3K pathways. Likewise, activation of the CB2 receptor by the agonist HU-308 (50 nM) for 16 hours induces the proliferation of BiB5 cells in culture, a lineage of rat neural progenitor cells, in a PI3K/AKT/mTORC1-dependent manner (Palazuelos et al. 2012). The same proliferative profile dependent on CB2 and mTORC1 activation is found in the ventricular and subventricular areas in embryonic cortical slices (E14.5) of mice (Palazuelos et al. 2012). Interestingly, oligodendrocyte progenitor proliferation is promoted by 2-AG through the PI3K/AKT/mTOR pathway (Gomez et al. 2015). Blockade of CB1 or CB2 and inhibition of 2-AG synthesis might regulate cell cycle exit via regulation of the cdk2/cyclin E association and p27 expression (Gomez et al. 2015).

CBD can also influence cell proliferation and migration in the hCMEC/D3 cell line. It is a microvascular endothelial cell line of the brain and represents a model of the blood-brain barrier. CBD at a concentration of 1 μM causes cell proliferation through activation of TRPV2. In addition, CBD also has an effect on cell motility by inducing cell migration in brain endothelial cells, and this effect was abrogated by using a TRPV2 antagonist. Finally, the study also found that CBD triggers tubulogenesis (Luo et al. 2019).

The brain is usually the main organ studied in the CNS. Most of the information about cannabinoids in development is related to it. However, there are other tissues that are being used to study the cannabinoid system, such as the retina. One study investigated the ontogenesis of CB1 and CB2 receptors during the development of chicken embryos. CB1 and CB2 expression was evaluated by western blot at E5, E7, E9, E14 and post-hatched day 7. In addition, expression of the MAGL enzyme was also visualized at all ages and in all layers of the retina (da Silva Sampaio et al. 2018). Later, we verified that the cannabinoid receptor agonists WIN-55,212-2 (0.5-1 μM), URB597 1 μM (FAAH inhibitor), and URB602 (50–100 μM, a MAGL inhibitor) inhibited the proliferation of retinal progenitors in culture. These data were corroborated by the lower incorporation of [^{3}H]-thymidine incorporation and, in the case of WIN, by the lower number of PCNA + cells in culture (Freitas et al. 2019). In rats, the presence of retinal CB1 receptor was first detected at E13, which is present throughout the rest of the developmental process (Buckley et al. 1997). During postnatal development, the CB2 receptor, FAAH, DAGLα and MAGL were also identified (Lu et al. 2000; Yazulla 2010). As cannabinoids cross the blood-brain barrier, it is very likely that they are able to cross the blood-retinal barrier. In the developmental context, these data indicate that exposure to cannabinoids during pregnancy could disrupt the correct proliferation of neural progenitors in the retina, which would ultimately lead to dysfunctions of the visual system in the adult individual.

4.1.3. DIFFERENTIATION:

The cannabinoid system also influences neuronal differentiation. The activation of CB1 by 2-AG (1 μM) and Δ^9-THC (3 μM) in cortical neurons derived from hiPSC cells leads to a reduction in neurite outgrowth after 24 hours of exposure in an ERK- and AKT-dependent manner (Shum et al. 2020). Likewise, neurons differentiated from hiPSCs in the presence of Δ^9-THC or synthetic cannabinoids (THJ-018 and EG-018) show functional abnormalities in voltage-dependent calcium channels when stimulated with extracellular potassium. Many of these neurons do not or are slow to respond to potassium stimulation (Miranda et al.

2020). This fact may have a direct implication on neuronal connectivity and functionality during CNS development. Accordingly, pregnant Sprague Dawley rats treated with 2 mg/kg/day Δ^9-THC (from gestational day 5 until gestational day 20) led to sensorimotor dysfunctions and hyperactivity in newborn male rats after acute stimulation with Δ^9-THC, and both behaviors were associated with an increase in dopamine release in the nucleus accumbens. In addition, dopaminergic neurons fired action potentials more frequently in a spontaneous or evoked manner, showing a depolarized membrane potential and a reduced voltage threshold (Frau et al. 2019). Prenatal exposure to Δ^9-THC also modifies dopamine responses in pups treated acutely with Δ^9-THC, with an increase in the frequency of spontaneous and evoked action potentials dependent on CB1 (Frau et al., 2019). Dopaminergic hyperfunction was also observed in dopaminergic neurons derived from hiPSCs treated throughout the differentiation period with low doses of AEA (1 μM). On the other hand, treatment with high concentrations of AEA (10 μM) or Δ^9-THC (10 μM) weakens the functionality of dopaminergic neurons, as indicated by the decrease in ionic currents and synaptic activity (Stanslowsky et al. 2017). *In utero* exposure to Δ^9-THC also leads to cognitive and behavioral impairment in adult rats, which is related to increased neuronal hyperexcitability and reduced number and dendritic complexity of cholecystokinin-expressing interneurons (Bara et al. 2018; de Salas-Quiroga et al. 2020; Vargish et al. 2017). Collectively, these results suggest that prenatal exposure to Δ^9-THC influences neural connectivity and excitability and modifies dopaminergic system functionality, altering the intrinsic properties of neurons and endowing them with a hyperexcitable phenotype, a clinical feature of several psychiatric disorders, such as schizophrenia. These alterations in the dopaminergic system could also probably lead to addiction and/or hyperactivity.

4.1.4. CELL DEATH:

Many studies address the role of cannabinoids in cell death in pathological conditions, mainly cancer and neurodegenerative diseases. However, few studies have aimed to verify the possible negative effects that cannabinoid modulation might induce during development. The use of marijuana has increased in recent years, and approximately 3.9% of pregnant women and 7.6% of nonpregnant women of reproductive age use marijuana (Brown et al. 2017). In addition to recreational use, marijuana has been administered among pregnant women for its antiemetic effect (Flament, Scius, and Thonon 2020). Thus, it is important to understand the risks that the drug might cause to the developing embryo, especially because it has been shown that Δ^9-THC can cross the placental barrier and thus reach the fetus (Alexandre et al. 2020).

Synthetic cannabinoids are often more potent than Δ^9-THC, causing several adverse effects, such as psychosis, arrhythmia, myocardial infarction, and even death (Drummer, Gerostamoulos, and Woodford 2019; Shanks and Behonick 2016). One study in six patients who used synthetic cannabinoids found major intoxication and later failures of multiple organ systems, including hepatic and renal failure and psychogenic effects in the brain (Armstrong, McCurdy, and Heavner 2019). Although the mechanisms related to all these effects have yet to be elucidated, it is possible that other noncannabinoid receptors might be involved, with the influence of serotoninergic, dopaminergic, GABAergic and glutamatergic

receptors (Giorgetti et al. 2020). Therefore, the literature regarding the effects of synthetic cannabinoid-induced death, as well as its mechanisms, has yet to be further investigated.

Δ^9- THC induces cell death in cortical neuron culture cells via CB1R, which is blocked by the CB1R antagonist AM251 and by pertussis toxin (PTX), a $G_{i/0}$ inhibitor. Furthermore, Δ^9-THC led to an increase in the release of cytochrome c and, consequently, activation of caspase-3 in a PTX-sensitive manner. These data indicate that Δ^9-THC-induced cell death involves the activation of CB1R through cytochrome c release and caspase-3 activation (Campbell 2001). Another study demonstrated that Δ^9-THC could be neurotoxic in hippocampal neurons using P1 Sprague Dawley rats. Δ^9-THC caused neuronal death via CB1R, an event blocked by inhibitors of phospholipase A2 (PLA2) and COX-2 inhibitors such as aspirin and indomethacin. Thus, it is suggested that Δ^9-THC increases arachidonic acid through PLA2, activating COX-2 and leading to the formation of ROS, causing neuronal death (Fig. 4) (Chan et al. 1998). A recent study demonstrated that a high concentration of CBD (10 μM) increases caspase 3 immunolabeling and cell death in human iPSCs (Miranda et al. 2020). As mentioned, CBD did not change Ki67 detection, a marker of the cell cycle, suggesting that the CBD effect is restricted to cell death. However, the combination of CBD and Δ^9-THC would have a broader effect on CNS development. These studies raise concerns about the consequences of cannabinoid exposure during pregnancy and CNS alterations.

Synthetic cannabinoids also influence CNS cell survival. Synthetic agonist CP-55,940, a full mix agonist for CB1 and CB2 receptors, is cytotoxic in forebrain cultures of mice at 15 days of gestation. Treatment with CP-55,940 decreased cell viability, leading to apoptosis through annexin-V and caspase-3. This effect was reduced in the presence of AM251, a CB1 receptor antagonist, but not by AM630 (CB2 receptor antagonist). Furthermore, AM630 did not cause cytotoxicity (Fig. 4) (Kenichi Tomiyama and Funada 2014). The same cytotoxic effect was observed in the NG 108 – 15 neuroblastoma cell line, with the agonists CP-55,940, CP-47,497 (specific for CB1R) and CP-47,497-C8 (found in spice herbal blends), as AM251 (but not AM630) suppressed some of these cytotoxic effects. Therefore, these data show a clear CB1R-mediated apoptosis in forebrain cultures and in the neuroblastoma lineage NG 108 – 15 (Kenichi Tomiyama and Funada 2011). Dorsal forebrain organoids derived from human induced pluripotent stem cells (hiPSCs) treated with WIN-55,212-2 were selectively enriched for cells exhibiting both cleaved PARP (one of several known cellular substrates of caspases) and DNA fragmentation, which occurs when endonucleases cleave chromatin into nucleosomal units and is therefore a marker of apoptosis (Notaras et al. 2021).

In the retinal model, WIN 55,212-2 induces cell death during development. In E7C2 (C2- second day of culture) chick embryo cultures, treatment with WIN (0.5, 1, 5 μM) induced a significant decrease in the number of living retinal cells in a dose-dependent manner (Freitas et al. 2019). This effect was inhibited by AM251 but not by AM630, indicating that the visualized cell death is mediated by CB1 and not CB2. WIN-induced cell death was completely inhibited by the selective antagonist for P2X7R, A438079. P2X is an ATP purinoreceptor that has been associated with cannabinoids, and specifically, P2X7 is more related to the effects of inflammation, cell death and pain. WIN induced an increase in ROS and mitochondrial

stress, which could be involved in the cell death response (Fig. 4) (Freitas et al. 2019). Thus, it was seen that P2X7R is related to WIN-induced death of retinal cells, corroborating a huge amount of data revealing the important role of P2X7 in cell death regulation (Kanellopoulos and Delarasse 2019). Interestingly, even though P2X7R mediated WIN-induced cell death, the regulation of cell proliferation (mentioned before) by WIN was P2X7R-independent, showing the electivity of P2X7R signaling to promote cell death. Although the involvement of P2X7 in cell death is well known, this was the first study showing the relationship of cannabinoid-induced cell death with the P2X7 receptor. The mechanisms involved in this event are still widely unknown. One possibility is that CB1 could induce ATP release, which, in turn, stimulates P2X7 receptors, allowing an intracellular calcium increase and an increase in ROS and mitochondrial stress, leading to cell death. However, whether dying cells are induced by P2X7 is unknown. In cultured astrocytes, as well as in microglia, P2X7 promotes an increase in 2-AG production by enhancing DAGL and inhibiting MAGL activity in a calcium-dependent manner (Witting et al. 2004). Therefore, another raised question relates to CB1 activation by WIN that could possibly initiate positive feedback that increases endocannabinoid availability in a deadly pathway. Finally, the data also showed a decrease in glial, but not neuronal, cell markers, with a decrease in proliferation and cell viability. Thus, glial progenitors could undergo a reduction in proliferation together with an increase in cell death, leading to an increase in the neuronal population. Therefore, it is possible that endocannabinoids could favor neuronal differentiation in the retina.

Apoptosis induced by cannabinoids in the CNS. CP-55,940 activates CB1R and causes apoptosis via caspase-3 in forebrain cultures. Δ9-THC, through CB1R, leads to cytochrome c release and caspase-3 activation, inducing apoptosis in cultured cortical neuron cells. Δ9-THC increases AA through PLA2, activating COX-2 and culminating in an increase in ROS, causing hippocampal neural death. P2X7 activation increases the activity of NAPE-PLD and DAGL, leading to an increase in 2-AG and AEA. 2-AG, AEA and WIN activate CB1R and CB2R signaling through P2X7R and cytoplasmic Ca^{2+}, causing an increase in superoxide and cytochrome c release and leading to apoptosis in the retina during development.

The relationship between endocannabinoids and cell death in several tissues, including the CNS, is still scarce. AEA induces apoptosis in lymphocytes, pheochromocytoma and neuroblastoma. It has also been shown that AEA, but not 2-AG, PEA or AA, induces cell death in PC12 cells, activating p38 MAPK, JNK and ERK1/2 through cytochrome c release. Cell death observed in PC12 cells is dependent on lipid rafts but not on cannabinoid receptors (CB1, CB2 or TRPV1) (Sarker and Maruyama 2003; Sarker et al. 2003). Interestingly, the same response was detected for C6, Neuro-2a, HEK 293, CHO, HVSM cells, Jurkat, HL-60 and nerve growth factor (NGF)-differentiated PC12 (d-PC12) cells. Another study found that AEA induces apoptosis in CHP100 neuroblastoma cells and in C6 glioma cells via TRPV1. Furthermore, in C6 cells but not in CHP100 cells, MβCD reduced AEA-induced apoptosis. It was also demonstrated that cholesterol depletion through MβCD enhanced the signaling and binding of the CB1 receptor, stimulating MAPK and causing the reduction of cytochrome c release from mitochondria, showing that this is probably the mechanism involved in the protection caused by MβCD in C6 cells (Bari et al. 2005).

CBD (10 μM) is also neurotoxic when used in neural progenitors, inducing cell death in hiPSC cultures after 21–22 days *in vitro*. A lower concentration of CBD (1 μM) was associated with a consistently lower density culture but did not significantly differ from the control for cell death, suggesting that it could affect the proliferation and/or survival of neuronal progenitors and differentiated neurons (Miranda et al. 2020).

Most studies focus more on the influence of cannabinoids on cell death by apoptosis, especially in cancer strains, and little is known about their effects on processes such as autophagy. Basal autophagy is necessary for the degradation and removal of damaged proteins and organelles from the cell itself via lysosomes, protecting against apoptosis in many situations (Galluzzi et al. 2018). Autophagy is essential for many developmental and physiological processes, and dysfunction is related to several diseases (Lia Costa et al. 2016). However, depending on the context, autophagy can promote apoptosis (Filomeni, De Zio, and Cecconi 2015). Increased accumulation of lipofuscin was described in the CA3 region of the hippocampus of CB1 receptor KO mice. The accumulation of this pigment is usually associated with age-related oxidative stress and deficits in autophagy. In agreement, upregulation of the levels of autophagy markers, such as p62 and LC3-II, was observed in CB1 KO mice. Therefore, it was shown in a study that CB1 receptor functionality affects lysosomal activity and autophagy (Piyanova et al. 2013). In a model of experimental autoimmune encephalomyelitis, the synthetic CB2 selective agonist HU-308 stimulated autophagy and inhibited the activation of the NLRP3 inflammasome, preserving the spinal cord of C57BL/6 mice (Shao et al. 2014).

There is also a possibility that the protective effects of the drug Sativex® (27 mg/ml THC + 25 mg/ml CBD) observed in $PK^{-/-}$/Tau VLW mice, a model for complex neurodegenerative diseases, might be related to an increase in autophagy. Sativex® improves behavior, dopamine metabolism, oxidative stress, glial function and tau/amyloid pathology through reduction of free radicals, increase in mitochondrial activity and stimulation of autophagy. Moreover, mutated tau overexpression may impair autophagy. Thus, some of these protective effects may be related to the increase in autophagy induction and to the possible improvement of the process of autophagosome-lysosome fusion or lysosomal degradative activity (Casarejos et al. 2013).

It is largely unknown whether cannabinoids might cause cell death by necrosis other than in cancer cells, especially in the CNS. AEA (25–100 μmol/L) induces cell death by necrosis, in addition to proliferation inhibition, in primary hepatic stellate cells of humans and rats. However, the AEA effect was not mediated by CB1 and CB2 receptors but was probably dependent on lipid rafts, as MβCD prevented AEA binding to primary hepatic stellate cells, in addition to inhibiting ROS formation, intracellular calcium release and cell death (Siegmund et al. 2005).

Cell death triggered by the activation of CB1 and CB2 receptors is well established in tumor cells and in deciduous tissue cells (Fonseca, Teixeira, and Correia-da-Silva 2017). Multiple mechanisms follow this event, which are dependent on autophagy, reticulum stress and oxidative stress. Evidence suggests that activation of cannabinoid receptors leads to cell death in developing CNS cells, although the mechanisms

remain unclear. In any case, these findings need further attention as future consequences of exposure to exogenous cannabinoids during crucial stages of embryonic development.

5. Conclusion

Endocannabinoids are important and abundant modulators in CNS physiology and development (Campos et al. 2017; Parker 2017). A great amount of work shows a neuroprotective effect associated with endocannabinoids through classical CB1 and CB2 receptors in different CNS pathologies. Accordingly, medicinal cannabinoids are turning into a recent reality in several countries. Moreover, a large number of studies have demonstrated the antitumoral effects against transformed cell lines (Garcia-Arencibia, Molina-Holgado, and Molina-Holgado 2019). However, as we hope to be clearly pointed out in the present review, knowledge about the role of cannabinoid substances in the early developmental CNS, especially for cell death, is still scarce. The signaling pathways have been widely explored in the oncological research field and even in the mature CNS in the context of pathological or physiological conditions. Although rare, the data available indicate that endocannabinoids regulate cell fate, proliferation, differentiation and cell death, actively participating in the formation of the mature CNS. Thus, exogenous cannabinoid exposure could impair CNS development, possibly leading to a dysfunctional nervous system. Therefore, during pregnancy, the use of recreational *cannabis* must be avoided, and the medicinal/synthetic cannabinoid field must consider all these elements.

References

Aguado, Tania et al. 2006. “The Endocannabinoid System Promotes Astroglial Differentiation by Acting on Neural Progenitor Cells.” *Journal of Neuroscience* 26(5): 1551–61. https://doi: 10.1523/JNEUROSCI.3101-05.2006.

Alexandre, João, Helena Carmo, Félix Carvalho, and João Pedro Silva. 2020. “Synthetic Cannabinoids and Their Impact on Neurodevelopmental Processes.” *Addiction Biology* 25(2): 1–12.https://doi.org/10.1111/adb.12824wileyonlinelibrary.com/journal/adb.

Almada, Marta, Patrícia Alves, et al. 2020. “Synthetic Cannabinoids JWH-018, JWH-122, UR-144 and the Phytocannabinoid THC Activate Apoptosis in Placental Cells.” *Toxicology Letters* 319(June 2019): 129–37. https://doi.org/10.1016/j.toxlet.2019.11.004.

Almada, Marta, Lia Costa, et al. 2020. “The Endocannabinoid 2-Arachidonoylglycerol Promotes Endoplasmic Reticulum Stress in Placental Cells.” *Reproduction* 160(2): 171–80.https://doi.org/10.1530/REP-19-0539.

Amlani, Aarti et al. 2017. “Maternal Cannabinoid Use Alters Cannabinoid (CB1) and Endothelin (ETB) Receptor Expression in the Brains of Dams but Not Their Offspring.” *Developmental Neuroscience* 39(6): 498–506.https://doi.org/10.1159/000480453.

Armstrong, Faith, Michael T. McCurdy, and Mojdeh S. Heavner. 2019. “Synthetic Cannabinoid-Associated Multiple Organ Failure: Case Series and Literature Review.” *Pharmacotherapy* 39(4): 508–13.https://doi.org/10.1002/phar.2241.

Baggelaar, Marc P., Mauro Maccarrone, and Mario van der Stelt. 2018. “2-Arachidonoylglycerol: A Signaling Lipid with Manifold Actions in the Brain.” *Progress in Lipid Research* 71(November 2017): 1–17. https://doi.org/10.1016/j.plipres.2018.05.002.

Bara, Anissa et al. 2018. “Sex-Dependent Effects of in Utero Cannabinoid Exposure on Cortical Function.” *eLife* 7: 1–31.https://doi.org/10.7554/eLife.36234.001.

Bari, Monica et al. 2005. “Lipid Rafts Control Signaling of Type-1 Cannabinoid Receptors in Neuronal Cells: Implications for Anandamide-Induced Apoptosis.” *Journal of Biological Chemistry* 280(13): 12212–20.https://doi.org/10.1074/jbc.M411642200.

Baron, Eric P. 2018. “Medicinal Properties of Cannabinoids, Terpenes, and Flavonoids in Cannabis, and Benefits in Migraine, Headache, and Pain: An Update on Current Evidence and Cannabis Science.” *Headache* 58(7): 1139–86.https://doi.org/10.1111/head.13345.

Basavarajappa, Balapal, Ralph Nixon, and Ottavio Arancio. 2009. “Endocannabinoid System: Emerging Role from Neurodevelopment to Neurodegeneration.” *Mini-Reviews in Medicinal Chemistry* 9(4): 448–62. https://doi: 10.2174/138955709787847921.

Bénard, Giovanni et al. 2012. “Mitochondrial CB 1 Receptors Regulate Neuronal Energy Metabolism.” *Nature Neuroscience* 15(4): 558–64.https://doi.org/10.1038/nn.3053.

Billakota, Santoshi, Orrin Devinsky, and Eric Marsh. 2019. “Cannabinoid Therapy in Epilepsy.” *Current Opinion in Neurology* 32(2): 220–26. https://doi: 10.1097/WCO.0000000000000660.

Bisogno, Tiziana et al. 2003. “Cloning of the First Sn1-DAG Lipases Points to the Spatial and Temporal Regulation of Endocannabinoid Signaling in the Brain.” *Journal of Cell Biology* 163(3): 463–68.https://doi.org/10.1083/jcb.200305129.

Blankman, Jacqueline L, Gabriel M Simon, and Benjamin F Cravatt. 2009. “A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol.” 14(12): 1347–56.https://doi: 10.1016/j.chembiol.2007.11.006.

Bonini, Sara Anna et al. 2018. “Cannabis Sativa: A Comprehensive Ethnopharmacological Review of a Medicinal Plant with a Long History.” *Journal of Ethnopharmacology* 227(May): 300–315. https://doi.org/10.1016/j.jep.2018.09.004.

Borrelli, Francesca et al. 2015. “Palmitoylethanolamide, a Naturally Occurring Lipid, Is an Orally Effective Intestinal Anti-Inflammatory Agent.” *British Journal of Pharmacology* 172(1): 142–58. https:// doi: 10.1111/bph.12907.

Brittis, Perry A., Jerry Silver, Frank S. Walsh, and Patrick Doherty. 1996. “Fibroblast Growth Factor Receptor Function Is Required for the Orderly Projection of Ganglion Cell Axons in the Developing Mammalian Retina.” *Molecular and Cellular Neurosciences* 8(2–3): 120–28. https://doi:10.1006/mcne.1996.0051.

Brown, A. J. 2007. “Novel Cannabinoid Receptors.” *British Journal of Pharmacology* 152(5): 567–75.https://doi.org/10.1038/sj.bjp.0707481.

Brown, Qiana L. et al. 2017. “Trends in Marijuana Use among Pregnant and Nonpregnant Reproductive-Aged Women, 2002-2014.” *JAMA - Journal of the American Medical Association* 317(2): 207–9. http://doi:10.1001/jama.2016.17383.

Buckley, N. E., S. Hansson, G. Harta, and É Mezey. 1997. “Expression of the CB1 and CB2 Receptor Messenger RNAs during Embryonic Development in the Rat.” *Neuroscience* 82(4): 1131–49.https://doi.org/10.1016/S0306-4522(97)00348-5.

Busquets-Garcia, Arnau, Jaideep Bains, and Giovanni Marsicano. 2018. “CB 1 Receptor Signaling in the Brain: Extracting Specificity from Ubiquity.” *Neuropsychopharmacology* 43(1): 4–20. http://dx.doi.org/10.1038/npp.2017.206.

Campbell, Veronica A. 2001. “Tetrahydrocannabinol-Induced Apoptosis of Cultured Cortical Neurones Is Associated with Cytochrome c Release and Caspase-3 Activation.” *Neuropharmacology* 40(5): 702–9.https://doi.org/10.1016/S0028-3908(00)00210-0.

Campos, Alline C. et al. 2017. “Cannabinoids as Regulators of Neural Development and Adult Neurogenesis.” : 117–36. http://link.springer.com/10.1007/978-3-319-49343-5.

Casarejos, Maria J. et al. 2013. “Natural Cannabinoids Improve Dopamine Neurotransmission and Tau and Amyloid Pathology in a Mouse Model of Tauopathy.” *Journal of Alzheimer’s Disease* 35(3): 525–39. https://doi 10.3233/JAD-130050

Chan, Guy Chiu Kai, Thomas R. Hinds, Soren Impey, and Daniel R. Storm. 1998. “Hippocampal Neurotoxicity of Δ9-Tetrahydrocannabinol.” *Journal of Neuroscience* 18(14): 5322–32.https://doi.org/10.1523/JNEUROSCI.18-14-05322.1998.

Chen, De Jie et al. 2017. “Brain Cannabinoid Receptor 2: Expression, Function and Modulation.” *Acta Pharmacologica Sinica* 38(3): 312–16. https://doi: 10.1038/aps.2016.149.

Coke, Christopher J. et al. 2016. “Simultaneous Activation of Induced Heterodimerization between CXCR4 Chemokine Receptor and Cannabinoid Receptor 2 (CB2) Reveals a Mechanism for Regulation of Tumor Progression.” *Journal of Biological Chemistry* 291(19): 9991–10005.https://doi.org/10.1074/jbc.M115.712661.

Corsi, Daniel J. et al. 2019. “Trends and Correlates of Cannabis Use in Pregnancy: A Population-Based Study in Ontario, Canada from 2012 to 2017.” *Canadian Journal of Public Health* 110(1): 76–84.https://doi.org/10.17269/s41997-018-0148-0.

Cortez, Isadora L., Naielly Rodrigues da Silva, Francisco S. Guimarães, and Felipe V. Gomes. 2020. “Are CB2 Receptors a New Target for Schizophrenia Treatment?” *Frontiers in Psychiatry* 11(October): 1–11. https://doi: 10.3389/fpsyt.2020.587154Schizophrenia.

Costa, L. et al. 2021. “The Major Endocannabinoid Anandamide (AEA) Induces Apoptosis of Human Granulosa Cells.” *Prostaglandins Leukotrienes and Essential Fatty Acids* 171(February): 102311. https://doi.org/10.1016/j.plefa.2021.102311.

Costa, Lia et al. 2016. “Cannabinoid-Induced Autophagy: Protective or Death Role?” *Prostaglandins and Other Lipid Mediators* 122: 54–63.https://doi.org/10.1016/j.prostaglandins.2015.12.006.

Cottone, Erika et al. 2021. “Cannabinoid Receptor Modulation of Neurogenesis: ST14A Striatal Neural Progenitor Cells as a Simplified in Vitro Model.” *Molecules* 26(5).https://doi.org/10.3390/molecules26051448.

Cravatt, Benjamin F. et al. 2001. “Supersensitivity to Anandamide and Enhanced Endogenous Cannabinoid Signaling in Mice Lacking Fatty Acid Amide Hydrolase.” *Proceedings of the National Academy of Sciences of the United States of America* 98(16): 9371–76.https://doi.org/10.1073/pnas.161191698.

Cristino, Luigia, Tiziana Bisogno, and Vincenzo Di Marzo. 2020. “Cannabinoids and the Expanded Endocannabinoid System in Neurological Disorders.” *Nature Reviews Neurology* 16(1): 9–29. http://dx.doi.org/10.1038/s41582-019-0284-z.

Das, Subhadip et al. 2019. “Cannabinoid Signaling in Cancer.” *Advances in Experimental Medicine and Biology* 1162: 51–61.https://doi.org/10.1007/978-3-030-21737-2_451.

Drummer, Olaf H., Dimitri Gerostamoulos, and Noel W. Woodford. 2019. “Cannabis as a Cause of Death: A Review.” *Forensic Science International* 298: 298–306. https://doi.org/10.1016/j.forsciint.2019.03.007.

Dyall, S. C. et al. 2016. “Distinctive Effects of Eicosapentaenoic and Docosahexaenoic Acids in Regulating Neural Stem Cell Fate Are Mediated via Endocannabinoid Signalling Pathways.” *Neuropharmacology* 107: 387–95. http://dx.doi.org/10.1016/j.neuropharm.2016.03.055.

Fernández-ruiz, Javier et al. 2000. “The endogenous cannabinoid system and brain development.” *Tins* 23(1). https://doi.org/10.1016/S0166-2236(99)01491-5.

Ferreira, Filipa Fiel et al. 2018. “Brain-Derived Neurotrophic Factor (BDNF) Role in Cannabinoid-Mediated Neurogenesis.” *Frontiers in Cellular Neuroscience* 12(November): 1–16. https://doi: 10.3389/fncel.2018.00441.

Filomeni, G., D. De Zio, and F. Cecconi. 2015. “Oxidative Stress and Autophagy: The Clash between Damage and Metabolic Needs.” *Cell Death and Differentiation* 22(3): 377–88. http://dx.doi.org/10.1038/cdd.2014.150.

Flament, Julien, Nathan Scius, and Henri Thonon. 2020. “Cannabinoid Hyperemesis Syndrome in the Pregnant Patient: Clinical Case and Literature Review.” *International Journal of Emergency Medicine* 13(1): 1–4.https://doi.org/10.1186/s12245-020-00311-y.

Fonseca, B. M. et al. 2010. “The Endocannabinoid 2-Arachidonoylglycerol (2-AG) and Metabolizing Enzymes during Rat Fetoplacental Development: A Role in Uterine Remodelling.” *International Journal of Biochemistry and Cell Biology* 42(11): 1884–92. http://dx.doi.org/10.1016/j.biocel.2010.08.006.

Fonseca, B. M., G. Correia-da-Silva, and N. A. Teixeira. 2009. “Anandamide-Induced Cell Death: Dual Effects in Primary Rat Decidual Cell Cultures.” *Placenta* 30(8): 686–92. http://dx.doi.org/10.1016/j.placenta.2009.05.012.

Fonseca, B. M., N. A. Teixeira, and G. Correia-da-Silva. 2017. “Cannabinoids as Modulators of Cell Death: Clinical Applications and Future Directions.” *Reviews of Physiology, Biochemistry and Pharmacology* 173: 63–88. https://doi: 10.1007/112_2017_3

Frau, Roberto et al. 2019. “Prenatal THC Exposure Produces a Hyperdopaminergic Phenotype Rescued by Pregnenolone.” *Nature Neuroscience* 22(12): 1975–85. https://doi:10.1038/s41593-019-0512-2.Prenatal

Freitas, Hércules Rezende et al. 2019. “Cannabinoids Induce Cell Death and Promote P2X7 Receptor Signaling in Retinal Glial Progenitors in Culture.” *Molecular Neurobiology* 56(9): 6472–86.https://doi.org/10.1007/s12035-019-1537-y.

Freund, Tamás F., István Katona, and Daniele Piomelli. 2003. “Role of Endogenous Cannabinoids in Synaptic Signaling.” *Physiological Reviews* 83(3): 1017–66. http://doi: 10.1152/physrev.00004.2003.

Galluzzi, Lorenzo et al. 2018. “Molecular Mechanisms of Cell Death: Recommendations of the Nomenclature Committee on Cell Death 2018.” *Cell Death and Differentiation* 25(3): 486–541.https://doi.org/10.1038/s41418-017-0012-4.

Galve-Roperh, Ismael et al. 2002. “Mechanism of Extracellular Signal-Regulated Kinase Activation by the CB1 Cannabinoid Receptor.” *Molecular Pharmacology* 62(6): 1385–92.https://doi.org/10.1124/mol.62.6.1385.

———. 2006. “Endocannabinoids: A New Family of Lipid Mediators Involved in the Regulation of Neural Cell Development.” *Current Pharmaceutical Design* 12(18): 2319–25. https://doi:10.2174/138161206777585139

———. 2013. “Cannabinoid Receptor Signaling in Progenitor/Stem Cell Proliferation and Differentiation.” *Progress in Lipid Research* 52(4): 633–50. http://dx.doi.org/10.1016/j.plipres.2013.05.004.

Garcia-Arencibia, Moises, Eduardo Molina-Holgado, and Francisco Molina-Holgado. 2019. “Effect of Endocannabinoid Signalling on Cell Fate: Life, Death, Differentiation and Proliferation of Brain Cells.” *British Journal of Pharmacology* 176(10): 1361–69.https://doi.org/10.1111/bph.14369.

García, María del Carmen, Edda Adler-Graschinsky, and Stella Maris Celuch. 2009. “Enhancement of the Hypotensive Effects of Intrathecally Injected Endocannabinoids by the Entourage Compound Palmitoylethanolamide.” *European Journal of Pharmacology* 610(1–3): 75–80. http://dx.doi.org/10.1016/j.ejphar.2009.03.021.

Giorgetti, Arianna et al. 2020. “Post-Mortem Toxicology: A Systematic Review of Death Cases Involving Synthetic Cannabinoid Receptor Agonists.” *Frontiers in Psychiatry* 11(May): 1–22. https://doi: 10.3389/fpsyt.2020.00464Post-Mortem

Gómez Del Pulgar, Teresa, Guillermo Velasco, and Manuel Guzmán. 2000. “The CB1 Cannabinoid Receptor Is Coupled to the Activation of Protein Kinase B/Akt.” *Biochemical Journal* 347(2): 369–73. https://doi: 10.1042/0264-6021:3470369

Gomez, Oscar et al. 2015. “A Basal Tone of 2-Arachidonoylglycerol Contributes to Early Oligodendrocyte Progenitor Proliferation by Activating Phosphatidylinositol 3-Kinase (PI3K)/AKT and the Mammalian Target of Rapamycin (MTOR) Pathways.” *Journal of Neuroimmune Pharmacology* 10(2): 309–17. https://doi: 10.1007/s11481-015-9609-x.

Grant, Kimberly S. et al. 2018. “Cannabis Use during Pregnancy: Pharmacokinetics and Effects on Child Development.” *Pharmacology and Therapeutics* 182(1): 133–51. https://doi:10.1016/j.pharmthera.2017.08.014.

Gulyas, A. I. et al. 2004. “Segregation of Two Endocannabinoid-Hydrolyzing Enzymes into Pre- and Postsynaptic Compartments in the Rat Hippocampus, Cerebellum and Amygdala.” *European Journal of Neuroscience* 20(2): 441–58. https://doi: 10.1111/j.1460-9568.2004.03428.x.

Guzmán, Manuel. 2003. “Cannabinoids: Potential Anticancer Agents.” *Nature Reviews Cancer* 3(10): 745–55. http://www.nature.com/doifinder/10.1038/nrc1188. https://doi:10.1038/nrc1188.

Han, Jing et al. 2012. “Acute Cannabinoids Impair Working Memory through Astroglial CB1 Receptor Modulation of Hippocampal LTD.” *Cell* 148(5): 1039–50. http://dx.doi.org/10.1016/j.cell.2012.01.037.

Hansen, Henrik H. et al. 2008. “Cannabinoids Enhance Susceptibility of Immature Brain to Ethanol Neurotoxicity.” *Annals of Neurology* 64(1): 42–52.https://doi.org/10.1002/ana.21287.

Hanuš, Lumír O. 2007. “Discovery and Isolation of Anandamide and Other Endocannabinoids.” *Chemistry and Biodiversity* 4(8): 1828–41.https://doi.org/10.1002/cbdv.200790154.

Harkany, Tibor et al. 2007. “The Emerging Functions of Endocannabinoid Signaling during CNS Development.” *Trends in Pharmacological Sciences* 28(2): 83–92.https://doi:10.1016/j.tips.2006.12.004.

Harkany, Tibor, Erik Keimpema, Klaudia Barabás, and Jan Mulder. 2008. “Endocannabinoid Functions Controlling Neuronal Specification during Brain Development.” *Molecular and Cellular Endocrinology* 286(1-2 SUPPL. 1): 84–90. https://doi:10.1016/j.mce.2008.02.011.

Heimann, Andrea S. et al. 2021. “Hemopressin as a Breakthrough for the Cannabinoid Field.” *Neuropharmacology* 183: 108406. https://doi.org/10.1016/j.neuropharm.2020.108406.

Huestis, Marilyn A. et al. 2019. “Cannabidiol Adverse Effects and Toxicity.” *Current Neuropharmacology* 17(10): 974–89. https://doi:10.2174/1570159X17666190603171901

Hutch, Chelsea R., and Colleen C. Hegg. 2016. “Cannabinoid Receptor Signaling Induces Proliferation but Not Neurogenesis in the Mouse Olfactory Epithelium.” *Neurogenesis* 3(1): 1–15. http://dx.doi.org/10.1080/23262133.2015.1118177.

Jiang, Wen et al. 2005. “Cannabinoids Promote Embryonic and Adult Hippocampus Neurogenesis and Produce Anxiolytic- and Antidepressant-like Effects.” *Journal of Clinical Investigation* 115(11): 3104–16. https://doi:10.1172/JCI25509.

Kaczocha, Martin et al. 2010. “Lipid Droplets Are Novel Sites of N-Acylethanolamine Inactivation by Fatty Acid Amide Hydrolase-2.” *Journal of Biological Chemistry* 285(4): 2796–2806.https://doi.org/10.1074/jbc.M109.058461.

Kanellopoulos, Jean M., and Cécile Delarasse. 2019. “Pleiotropic Roles of P2X7 in the Central Nervous System.” *Frontiers in Cellular Neuroscience* 13(September): 1–18. http://doi: 10.3389/fncel.2019.00401

Keimpema, Erik et al. 2010. “Differential Subcellular Recruitment of Monoacylglycerol Lipase Generates Spatial Specificity of 2-Arachidonoyl Glycerol Signaling during Axonal Pathfinding.” *Journal of Neuroscience* 30(42): 13992–7. https://doi: 10.1523/JNEUROSCI.2126-10.2010.

Kendall, Debra A., and Guillermo A. Yudowski. 2017. “Cannabinoid Receptors in the Central Nervous System: Their Signaling and Roles in Disease.” *Frontiers in Cellular Neuroscience* 10(January): 1–10. http://doi: 10.3389/fncel.2016.00294Edited.

Koltai, Hinanit, and Dvora Namdar. 2020. “Cannabis Phytomolecule ‘Entourage’: From Domestication to Medical Use.” *Trends in Plant Science* 25(10): 976–84. https://doi.org/10.1016/j.tplants.2020.04.007.

Lee, Jong Eun et al. 2011. “Autophagy Regulates Embryonic Survival during Delayed Implantation.” *Endocrinology* 152(5): 2067–75. https://doi: 10.1210/en.2010-1456.

Leonelli, Mauro, Luiz R.G. Britto, Gabriela P. Chaves, and Andréa S. Torrão. 2005. “Developmental Expression of Cannabinoid Receptors in the Chick Retinotectal System.” *Developmental Brain Research* 156(2): 176–82.https://doi.org/10.1016/j.devbrainres.2005.02.009.

Levinsohn, Erik A., and Kevin P. Hill. 2020. “Clinical Uses of Cannabis and Cannabinoids in the United States.” *Journal of the Neurological Sciences* 411(August 2019): 116717. https://doi.org/10.1016/j.jns.2020.116717.

Lojpur, Tina et al. 2019. “Δ9-Tetrahydrocannabinol Leads to Endoplasmic Reticulum Stress and Mitochondrial Dysfunction in Human BeWo Trophoblasts.” *Reproductive Toxicology* 87(May): 21–31. https://doi.org/10.1016/j.reprotox.2019.04.008.

López-Moreno, José Antonio, Gustavo González-Cuevas, Guillermo Moreno, and Miguel Navarro. 2008. “The Pharmacology of the Endocannabinoid System: Functional and Structural Interactions with Other Neurotransmitter Systems and Their Repercussions in Behavioral Addiction.” *Addiction Biology* 13(2): 160–87. http://doi:10.1111/j.1369-1600.2008.00105.x.

Lu, Qingjun, Alex Straiker, Qingxian Lu, and Greg Maguire. 2000. “Expression of CB2 Cannabinoid Receptor MRNA in Adult Rat Retina.” *Visual Neuroscience* 17(1): 91–95.https://doi.org/10.1017/S0952523800171093.

Luo, Huilong et al. 2019. “Cannabidiol Increases Proliferation, Migration, Tubulogenesis, and Integrity of Human Brain Endothelial Cells through TRPV2 Activation.” *Molecular Pharmaceutics* 16(3): 1312–26. https://doi: 10.1021/acs.molpharmaceut.8b01252.

Maccarrone, Mauro et al. 2015. “Endocannabinoid Signaling at the Periphery: 50 Years after THC.” *Trends in Pharmacological Sciences* 36(5): 277–96. http://doi:10.1016/j.tips.2015.02.008.

Maccarrone. 2017. “Metabolism of the Endocannabinoid Anandamide: Open Questions after 25 Years.” *Frontiers in Molecular Neuroscience* 10(May): 1–10. https://doi: 10.3389/fnmol.2017.00166Metabolism

Maccarrone, Mauro et al. 2014. “Programming of Neural Cells by (Endo)Cannabinoids: From Physiological Rules to Emerging Therapies.” *Nature Reviews Neuroscience* 15(12): 786–801. http://www.nature.com/articles/nrn3846 (June 20, 2018). https://doi:10.1038/nrn3846.

Mackie, K. 2005. “Distribution of Cannabinoid Receptors in the Central and Peripheral Nervous System.” *Handbook of Experimental Pharmacology* 168: 299–325. https://doi:10.1007/3-540-26573-2_10.

Maia, J., B. M. Fonseca, N. Teixeira, and G. Correia-Da-Silva. 2020. “The Fundamental Role of the Endocannabinoid System in Endometrium and Placenta: Implications in Pathophysiological Aspects of Uterine and Pregnancy Disorders.” *Human Reproduction Update* 26(4): 586–602. https://doi:10.1093/humupd/dmaa005The

Martínez-Pinilla, E. et al. 2014. “CB1 and GPR55 Receptors Are Co-Expressed and Form Heteromers in Rat and Monkey Striatum.” *Experimental Neurology* 261: 44–52. http://dx.doi.org/10.1016/j.expneurol.2014.06.017.

Mccoy, Kathleen L. 2016. “Interaction between Cannabinoid System and Toll-Like Receptors Controls Inflammation.” *Mediators of Inflammation* 2016.http://dx.doi.org/10.1155/2016/5831315.

Miranda, Cláudia C. et al. 2020. “HiPSC-Based Model of Prenatal Exposure to Cannabinoids: Effect on Neuronal Differentiation.” *Frontiers in Molecular Neuroscience* 13(July): 1–11. https://doi: 10.3389/fnmol.2020.00119.

Molina-Holgado, Eduardo et al. 2002. “Cannabinoids Promote Oligodendrocyte Progenitor Survival: Involvement of Cannabinoid Receptors and Phosphatidylinositol-3 Kinase/Akt Signaling.” *Journal of Neuroscience* 22(22): 9742–53.https://doi.org/10.1523/JNEUROSCI.22-22-09742.2002.

Morales, Paula, and Patricia H. Reggio. 2017. “ An Update on Non-CB 1 , Non-CB 2 Cannabinoid Related G-Protein-Coupled Receptors .” *Cannabis and Cannabinoid Research* 2(1): 265–73. https://doi: 10.1089/can.2017.0036.

Muccioli, Giulio G. et al. 2007. “Identification of a Novel Endocannabinoid-Hydrolyzing Enzyme Expressed by Microglial Cells.” *Journal of Neuroscience* 27(11): 2883–89.https://doi.org/10.1523/JNEUROSCI.4830-06.2007.

Muller, Chanté, Paula Morales, and Patricia H. Reggio. 2019. “Cannabinoid Ligands Targeting TRP Channels.” *Frontiers in Molecular Neuroscience* 11(January): 1–15. https://doi: 10.3389/fnmol.2018.00487Cannabinoid.

Murataeva, N., A. Straiker, and K. MacKie. 2014. “Parsing the Players: 2-Arachidonoylglycerol Synthesis and Degradation in the CNS.” *British Journal of Pharmacology* 171(6): 1379–91.https://doi: 10.1111/bph.12411.

Navarrete, Francisco et al. 2020. “Cannabis Use in Pregnant and Breastfeeding Women: Behavioral and Neurobiological Consequences.” *Frontiers in Psychiatry* 11(November): 1–15. https://doi: 10.3389/fpsyt.2020.586447Cannabis.

Notaras, Michael et al. 2021. “Neurodevelopmental Signatures of Narcotic and Neuropsychiatric Risk Factors in 3D Human-Derived Forebrain Organoids.” *Molecular Psychiatry* (June). http://dx.doi.org/10.1038/s41380-021-01189-9.

Nyilas, Rita et al. 2008. “Enzymatic Machinery for Endocannabinoid Biosynthesis Associated with Calcium Stores in Glutamatergic Axon Terminals.” *Journal of Neuroscience* 28(5): 1058–63.https://doi: 10.1523/JNEUROSCI.5102-07.2008.

O’Sullivan, Saoirse Elizabeth. 2016. “An Update on PPAR Activation by Cannabinoids.” *British Journal of Pharmacology* 173(12): 1899–1910.https://doi.org/10.1111/bph.13497.

Oh, Hyun Ah et al. 2013. “Uncovering a Role for Endocannabinoid Signaling in Autophagy in Preimplantation Mouse Embryos.” *Molecular Human Reproduction* 19(2): 93–

101.https://doi:10.1093/molehr/gas049.

Pacher, Pal, Natalya M. Kogan, and Raphael Mechoulam. 2020. “Beyond THC and Endocannabinoids.” *Annual Review of Pharmacology and Toxicology* 60: 637–59.https://doi.org/10.1146/annurev-pharmtox-010818-021441.

Palazuelos, Javier et al. 2012. “CB 2 Cannabinoid Receptors Promote Neural Progenitor Cell Proliferation via MTORC1 Signaling.” *Journal of Biological Chemistry* 287(2): 1198–1209.https://doi.org/10.1074/jbc.M111.291294.

Paraíso-Luna, Juan et al. 2020. “Endocannabinoid Signalling in Stem Cells and Cerebral Organoids Drives Differentiation to Deep Layer Projection Neurons via CB1 Receptors.” *Development (Cambridge)* 147(24).https://doi.org/10.1242/dev.192161.

Paria, B. C. et al. 2001. “Dysregulated Cannabinoid Signaling Disrupts Uterine Receptivity for Embryo Implantation.” *Journal of Biological Chemistry* 276(23): 20523–28.https://doi.org/10.1074/jbc.M100679200.

Parker, Linda A. 2017. Cannabinoids and the Brain *Cannabinoids and the Brain*.

Peres, Fernanda F. et al. 2018. “Cannabidiol as a Promising Strategy to Treat and Prevent Movement Disorders?” *Frontiers in Pharmacology* 9(MAY): 1–12. https://doi: 10.3389/fphar.2018.00482Cannabidiol.

Pertwee, R. 2010. “Receptors and Channels Targeted by Synthetic Cannabinoid Receptor Agonists and Antagonists.” *Current Medicinal Chemistry* 17(14): 1360–81.https://doi: 10.2174/092986710790980050.

Pertwee, Roger G. 2006. “Cannabinoid Pharmacology: The First 66 Years.” *British Journal of Pharmacology* 147(SUPPL. 1). http://doi: 10.1038/sj.bjp.0706406.

Philippot, Gaëtan et al. 2019. “A Single Δ9-Tetrahydrocannabinol (THC) Dose during Brain Development Affects Markers of Neurotrophy, Oxidative Stress, and Apoptosis.” *Frontiers in Pharmacology* 10(October): 1–9. http://doi: 10.3389/fphar.2019.01156ª.

Pinho Costa, José Luis G. et al. 2011. “Neurobiology of Cannabis: From the Endocannabinoid System to Cannabis-Related Disorders.” *Jornal Brasileiro de Psiquiatria* 60(2): 110–22.https://doi.org/10.1590/S0047-20852011000200006.

Piyanova, Anastasia et al. 2013. “Loss of CB1 Receptors Leads to Decreased Cathepsin D Levels and Accelerated Lipofuscin Accumulation in the Hippocampus.” *Mechanisms of Ageing and Development* 134(9): 391–99. http://dx.doi.org/10.1016/j.mad.2013.08.001.

Prenderville, Jack A., Áine M. Kelly, and Eric J. Downer. 2015. “The Role of Cannabinoids in Adult Neurogenesis.” *British Journal of Pharmacology* 172(16): 3950–63.https://doi: 10.1111/bph.13186.

Psychoyos, Delphine, Basalingappa Hungund, Thomas Cooper, and Richard H. Finnell. 2008. “A Cannabinoid Analogue of Δ9-Tetrahydrocannabinol Disrupts Neural Development in Chick.” *Birth Defects Research Part B - Developmental and Reproductive Toxicology* 83(5): 477–88.https://doi.org/10.1002/bdrb.20166.

Redmond, William John et al. 2014. “Ligand Determinants of Fatty Acid Activation of the Pronociceptive Ion Channel TRPA1.” *PeerJ* 2014(1): 1–20.https://doi: 10.7717/peerj.248.

Reggio, Patricia. 2010. “Endocannabinoid Binding to the Cannabinoid Receptors: What Is Known and What Remains Unknown.” *Current Medicinal Chemistry* 17(14): 1468–86. http://doi:10.2174/092986710790980005.

Riquelme-Sandoval, Agustín, Caio O. de Sá-Ferreira, Leo M. Miyakoshi, and Cecilia Hedin-Pereira. 2020. “New Insights Into Peptide Cannabinoids: Structure, Biosynthesis and Signaling.” *Frontiers in Pharmacology* 11(December).https://doi: 10.3389/fphar.2020.596572New.

Rodrigues, Rui S. et al. 2017. “Interaction between Cannabinoid Type 1 and Type 2 Receptors in the Modulation of Subventricular Zone and Dentate Gyrus Neurogenesis.” *Frontiers in Pharmacology* 8(AUG): 1–26. https://doi: 10.3389/fphar.2017.00516.

Rog, David J., Turo J. Nurmikko, and Carolyn A. Young. 2007. “Oromucosal Δ9-Tetrahydrocannabinol/Cannabidiol for Neuropathic Pain Associated with Multiple Sclerosis: An Uncontrolled, Open-Label, 2-Year Extension Trial.” *Clinical Therapeutics* 29(9): 2068–79. https:doi: 10.1016/j.clinthera.2007.09.013.

Rueda, Daniel et al. 2002. “The Endocannabinoid Anandamide Inhibits Neuronal Progenitor Cell Differentiation through Attenuation of the Rap1/B-Raf/ERK Pathway.” *Journal of Biological Chemistry* 277(48): 46645–50.https://doi.org/10.1074/jbc.M206590200.

Russo, Ethan B. 2019. “The Case for the Entourage Effect and Conventional Breeding of Clinical Cannabis: No ‘Strain,’ No Gain.” *Frontiers in Plant Science* 9(January): 1–8. https://doi: 10.3389/fpls.2018.01969.

Saario, Susanna M. et al. 2005. “Characterization of the Sulfhydryl-Sensitive Site in the Enzyme Responsible for Hydrolysis of 2-Arachidonoyl-Glycerol in Rat Cerebellar Membranes.” *Chemistry and Biology* 12(6): 649–56. https://doi:10.1016/j.chembiol.2005.04.013.

de Salas-Quiroga, Adán et al. 2020. “Long-Term Hippocampal Interneuronopathy Drives Sex-Dimorphic Spatial Memory Impairment Induced by Prenatal THC Exposure.” *Neuropsychopharmacology* 45(5): 877–86. http://dx.doi.org/10.1038/s41386-020-0621-3.

De Salas-Quiroga, Adán et al. 2015. “Prenatal Exposure to Cannabinoids Evokes Long-Lasting Functional Alterations by Targeting CB1 Receptors on Developing Cortical Neurons.” *Proceedings of the National*

Academy of Sciences of the United States of America 112(44): 13693–98.https://doi.org/10.1073/pnas.1514962112.

Sarker, K., and I. Maruyama. 2003. “Anandamide Induces Cell Death Independently of Cannabinoid Receptors or Vanilloid Receptor 1: Possible Involvement of Lipid Rafts.” *Cellular and Molecular Life Sciences* 60(6): 1200–1208.

Sarker, Krishna Pada et al. 2003. “ASK1-P38 MAPK/JNK Signaling Cascade Mediates Anandamide-Induced PC12 Cell Death.” *Journal of Neurochemistry* 85(1): 50–61.https://doi:10.1046/j.1471-4159.2003.01663.x.

Sarris, Jerome et al. 2020. “Medicinal Cannabis for Psychiatric Disorders: A Clinically-Focused Systematic Review.” *BMC Psychiatry* 20(1): 1–14.https://doi.org/10.1186/s12888-019-2409-8.

Schwitzer, Thomas et al. 2016. “The Endocannabinoid System in the Retina: From Physiology to Practical and Therapeutic Applications.” *Neural Plasticity* 2016.http://dx.doi.org/10.1155/2016/2916732.

Shanks, Kevin G., and George S. Behonick. 2016. “Death after Use of the Synthetic Cannabinoid 5F-AMB.” *Forensic Science International* 262: e21–24. http://dx.doi.org/10.1016/j.forsciint.2016.03.004.

Shao, Bo Zong et al. 2014. “Activating Cannabinoid Receptor 2 Alleviates Pathogenesis of Experimental Autoimmune Encephalomyelitis via Activation of Autophagy and Inhibiting NLRP3 Inflammasome.” *CNS Neuroscience and Therapeutics* 20(12): 1021–28. https://doi: 10.1111/cns.12349

Sharir, Haleli, and Mary E. Abood. 2010. “Pharmacological Characterization of GPR55, a Putative Cannabinoid Receptor.” *Pharmacology and Therapeutics* 126(3): 301–13. https://doi:10.1016/j.pharmthera.2010.02.004.

Shum, Carole et al. 2020. “Δ9-Tetrahydrocannabinol and 2-AG Decreases Neurite Outgrowth and Differentially Affects ERK1/2 and Akt Signaling in HiPSC-Derived Cortical Neurons.” *Molecular and Cellular Neuroscience* 103(December 2019): 103463. https://doi.org/10.1016/j.mcn.2019.103463.

Siegmund, Sören V. et al. 2005. “Anandamide Induces Necrosis in Primary Hepatic Stellate Cells.” *Hepatology* 41(5): 1085–95.https://doi.org/10.1002/hep.20667.

da Silva Sampaio, Luzia et al. 2018. “Cannabinoid Receptor Type 1 Expression in the Developing Avian Retina: Morphological and Functional Correlation with the Dopaminergic System.” *Frontiers in Cellular Neuroscience* 12(March): 1–10. https://doi: 10.3389/fncel.2018.00058.

Solymosi, Katalin, and Attila Kofalvi. 2016. 17 Mini-Reviews in Medicinal Chemistry *Cannabis: A Treasure Trove or Pandora’s Box?.*https://doi: 10.2174/1389557516666161004162133.

Stanslowsky, Nancy et al. 2017. “Functional Effects of Cannabinoids during Dopaminergic Specification of Human Neural Precursors Derived from Induced Pluripotent Stem Cells.” *Addiction Biology* 22(5):

1329–42.https://doi.org/10.1111/adb.12394.

Stott, C. G. et al. 2013. “A Phase i Study to Assess the Single and Multiple Dose Pharmacokinetics of THC/CBD Oromucosal Spray.” *European Journal of Clinical Pharmacology* 69(5): 1135–47.https://doi: 10.1007/s00228-012-1441-0.

Sugiura, Takayuki et al. 1996. “2-Arachidonylglycerol, a Putative Endogenous Cannabinoid Receptor Ligand, Induces Rapid, Transient Elevation of Intracellular Free Ca2+ Neuroblastoma X Glioma Hybrid NG108-15 Cells.” *Biochemical and Biophysical Research Communications* 229(1): 58–64.https://doi.org/10.1006/bbrc.1996.1757.

Taylor, Lesley et al. 2018. “A Phase I, Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose, Multiple Dose, and Food Effect Trial of the Safety, Tolerability and Pharmacokinetics of Highly Purified Cannabidiol in Healthy Subjects.” *CNS Drugs* 32(11): 1053–67. https://doi.org/10.1007/s40263-018-0578-5.

Tayo, Bola, Lesley Taylor, Farhad Sahebkar, and Gilmour Morrison. 2020. “A Phase I, Open-Label, Parallel-Group, Single-Dose Trial of the Pharmacokinetics, Safety, and Tolerability of Cannabidiol in Subjects with Mild to Severe Renal Impairment.” *Clinical Pharmacokinetics* 59(6): 747–55. https://doi.org/10.1007/s40262-019-00841-6.

Tomiyama, Ken ichi, and Masahiko Funada. 2014. “Cytotoxicity of Synthetic Cannabinoids on Primary Neuronal Cells of the Forebrain: The Involvement of Cannabinoid CB1 Receptors and Apoptotic Cell Death.” *Toxicology and Applied Pharmacology* 274(1): 17–23. http://dx.doi.org/10.1016/j.taap.2013.10.028.

Tomiyama, Kenichi, and Masahiko Funada. 2011. “Cytotoxicity of Synthetic Cannabinoids Found in ‘Spice’ Products: The Role of Cannabinoid Receptors and the Caspase Cascade in the NG 108-15 Cell Line.” *Toxicology Letters* 207(1): 12–17. http://dx.doi.org/10.1016/j.toxlet.2011.08.021.

Trabucco, E. et al. 2009. “Endocannabinoid System in First Trimester Placenta: Low FAAH and High CB1 Expression Characterize Spontaneous Miscarriage.” *Placenta* 30(6): 516–22. http://dx.doi.org/10.1016/j.placenta.2009.03.015.

Trazzi, Stefania et al. 2010. “CB1 Cannabinoid Receptors Increase Neuronal Precursor Proliferation through AKT/Glycogen Synthase Kinase-3β/β-Catenin Signaling.” *Journal of Biological Chemistry* 285(13): 10098–109.https://doi.org/10.1074/jbc.M109.043711.

Tsuboi, Kazuhito, Toru Uyama, Yasuo Okamoto, and Natsuo Ueda. 2018. “Endocannabinoids and Related N-Acylethanolamines: Biological Activities and Metabolism Makoto Murakami.” *Inflammation and Regeneration* 38(1): 1–10.https://doi.org/10.1186/s41232-018-0086-5.

Turu, Gábor, and László Hunyady. 2010. “Signal Transduction of the CB1 Cannabinoid Receptor.” *Journal of Molecular Endocrinology* 44(2): 75–85.https://doi.org/10.1677/JME-08-0190

Ueda, Natsuo, Kazuhito Tsuboi, and Toru Uyama. 2013. “Metabolism of Endocannabinoids and Related N-Acylethanolamines: Canonical and Alternative Pathways.” *FEBS Journal* 280(9): 1874–94. https://doi:10.1111/febs.12152.

Vargish, G. A. et al. 2017. “Persistent Inhibitory Circuit Defects and Disrupted Social Behaviour Following in Utero Exogenous Cannabinoid Exposure.” *Molecular Psychiatry* 22(1): 56–67. https://doi:10.1038/mp.2016.17.

Viñals, Xavier et al. 2015. “Cognitive Impairment Induced by Delta9- Tetrahydrocannabinol Occurs through Heteromers between Cannabinoid CB1 and Serotonin 5-HT2A Receptors.” *PLoS Biology* 13(7): 1–40. https://doi:10.1371/journal.pbio.1002194.

Wang, Haibin et al. 2007. “Differential Regulation of Endocannabinoid Synthesis and Degradation in the Uterus during Embryo Implantation.” *Prostaglandins and Other Lipid Mediators* 83(1–2): 62–74. https://doi:10.1016/j.prostaglandins.2006.09.009.

Wei, Binqing Q. et al. 2006. “A Second Fatty Acid Amide Hydrolase with Variable Distribution among Placental Mammals.” *Journal of Biological Chemistry* 281(48): 36569–78.https://doi.org/10.1074/jbc.M606646200.

Witting, Anke et al. 2004. “P2X7 Receptors Control 2-Arachidonoylglycerol Production by Microglial Cells.” *Proceedings of the National Academy of Sciences of the United States of America* 101(9): 3214–19. https://doi:10.1073/pnas.0306707101.

Xapelli, Sara et al. 2013. “Activation of Type 1 Cannabinoid Receptor (CB1R) Promotes Neurogenesis in Murine Subventricular Zone Cell Cultures.” *PLoS ONE* 8(5). https://doi:10.1371/journal.pone.0063529.

Yazulla, S. 2010. “Retinal Cannabinoids.” *Encyclopedia of the Eye* (July 2016): 45–53.http://dx.doi.org/10.1016/B978-0-12-809324-5.01504-2.

Young-Wolff, Kelly C. et al. 2017. “Trends in Self-Reported and Biochemically Tested Marijuana Use among Pregnant Females in California from 2009-2016.” *JAMA - Journal of the American Medical Association* 318(24): 2490–91. https://doi:10.1001/jama.2017.17225.

Zou, Shenglong, and Ujendra Kumar. 2018. “Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System.” *International Journal of Molecular Sciences* 19(3). https://doi:10.3390/ijms19030833.

Zou, Shenglong, Rishi K. Somvanshi, and Ujendra Kumar. 2017. “Somatostatin Receptor 5 Is a Prominent Regulator of Signaling Pathways in Cells with Coexpression of Cannabinoid Receptors 1.” *Neuroscience* 340(November): 218–31. http://dx.doi.org/10.1016/j.neuroscience.2016.10.056.

Zurolo, E. et al. 2010. “CB1 and CB2 Cannabinoid Receptor Expression during Development and in Epileptogenic Developmental Pathologies.” *Neuroscience* 170(1): 28–41. http://dx.doi.org/10.1016/j.neuroscience.2010.07.004.

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Figures

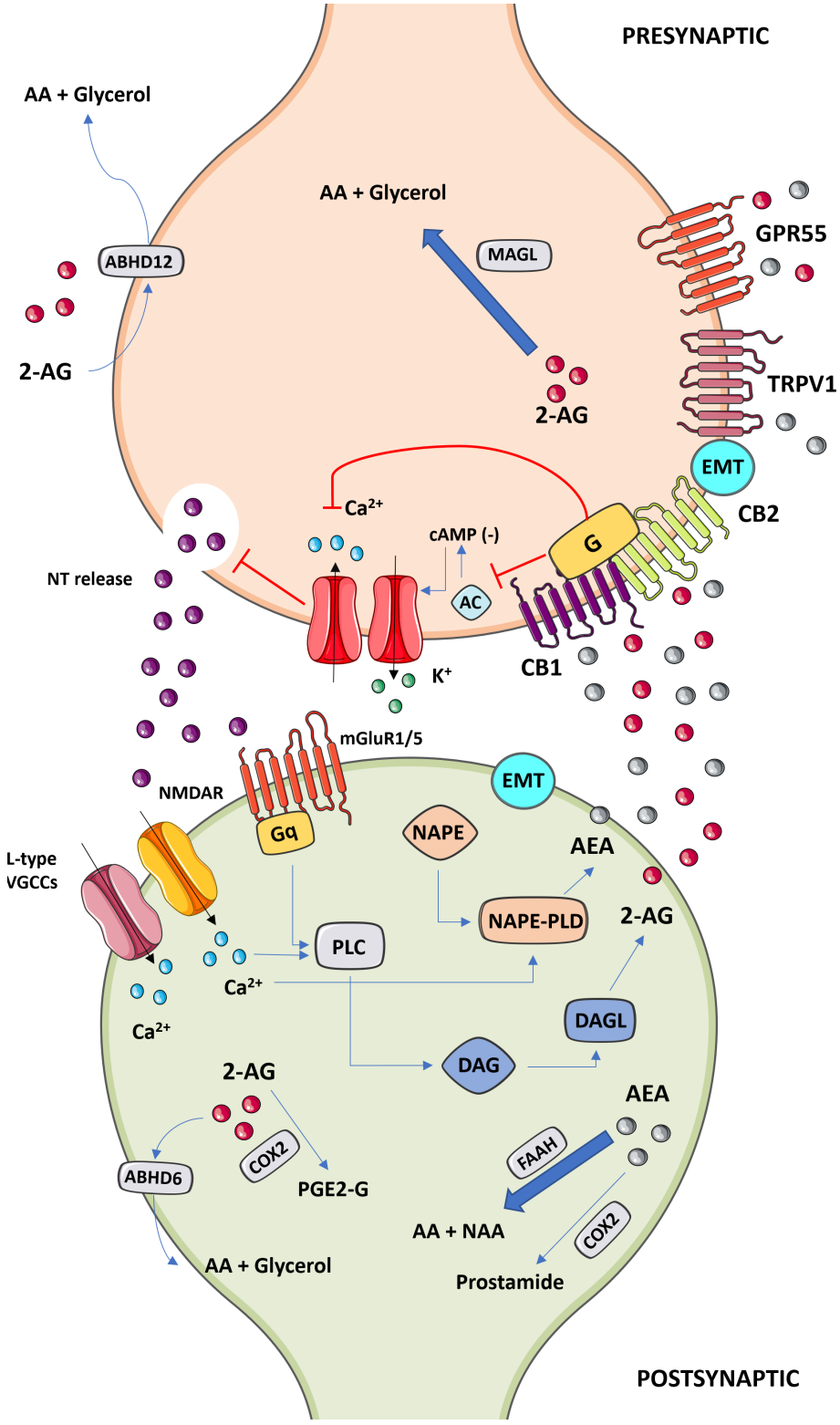

Figure 1

Signaling in the endocannabinoid synapse. Glutamate released by presynaptic cells stimulates ionotropic and metabotropic receptors in postsynaptic cells, leading to an increase in Ca^{2+} influx/concentration. Ca^{2+} and/or Gq stimulate PLC, which leads to the production of DAG, the substrate for DAGL to form 2-AG, while NAPE-PLD hydrolyses NAPE to form AEA. Endocannabinoids diffuse through the synaptic cleft, reaching the CB1 and CB2 receptors TRPV1 and GPR55, among others. The classical downstream

pathway of the CB receptor occurs through Gi-protein: inhibition of AC activity, modulation of K^+ and Ca^{2+} channels, and subsequent inhibition of NT release. The signaling is ended by endocannabinoid degradation. FAAH metabolizes AEA into AA and ethanolamine, while COX-2 can eventually generate prostamide from AEA. MAGL, ABHD12, and ABHD6 degrade 2-AG in AA and glycerol, while COX-2 is metabolized in PGE2-G. The thickness of arrows from metabolizing enzymes represents the importance in the control of AEA and 2-AG availability.

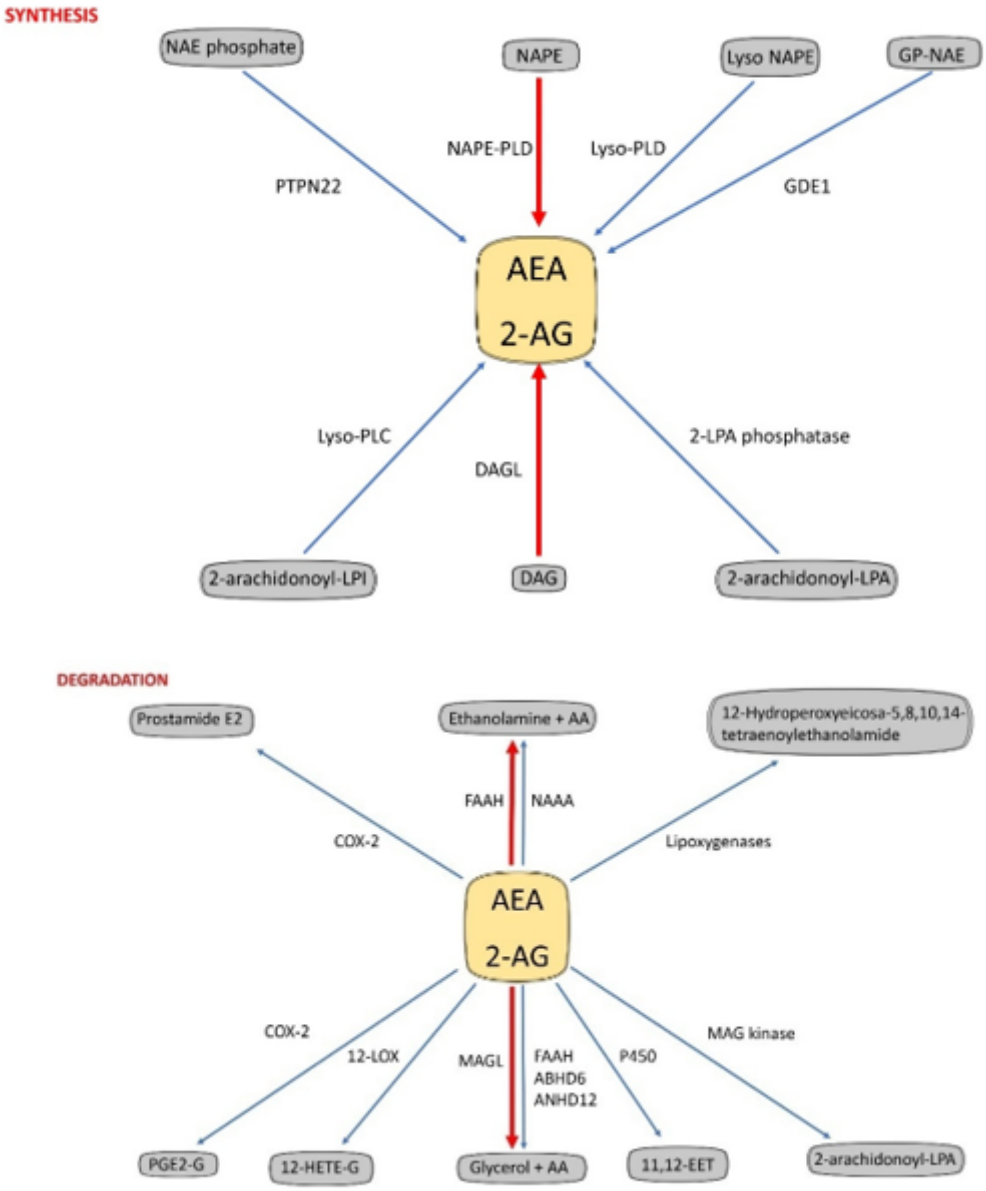

Figure 2

Primary metabolic pathways of AEA and 2-AG synthesis and degradation. AEA synthesis – An increase in Ca^{2+} stimulates NAPE-PLD to hydrolyze NAPE, producing AEA. PLC catalyzes the formation of phospho-

NAE, and the phosphatases PTPN22 and INPP5D then convert phospho-NAE to AEA. NAPE is catalyzed by sPLA2 or ABHD4, generating lyso-NAPE. Then, ABHD4 catalyzes the formation of GP-NAE, which is converted to AEA by the phosphodiesterase GDE1. 2-AG synthesis – PLC mediates the hydrolysis of PIP_2, which produces DAG, which is converted to 2-AG by DAGLα/β. Phosphatidic acid (PA) is hydrolyzed by PA phosphohydrolase, producing DAG, which is converted by DAGLα/β into 2-AG. AEA degradation – AEA is degraded primarily by FAAH into AA and ethanolamine, while COX-2 degrades into prostamide. 2-AG degradation – 2-AG is degraded primarily by MAGL into AA and glycerol. ABHD6 and ABHD12 also degrade 2-AG into AA and glycerol, although they are less common. COX-2 degrades 2-AG into PGE2-G.

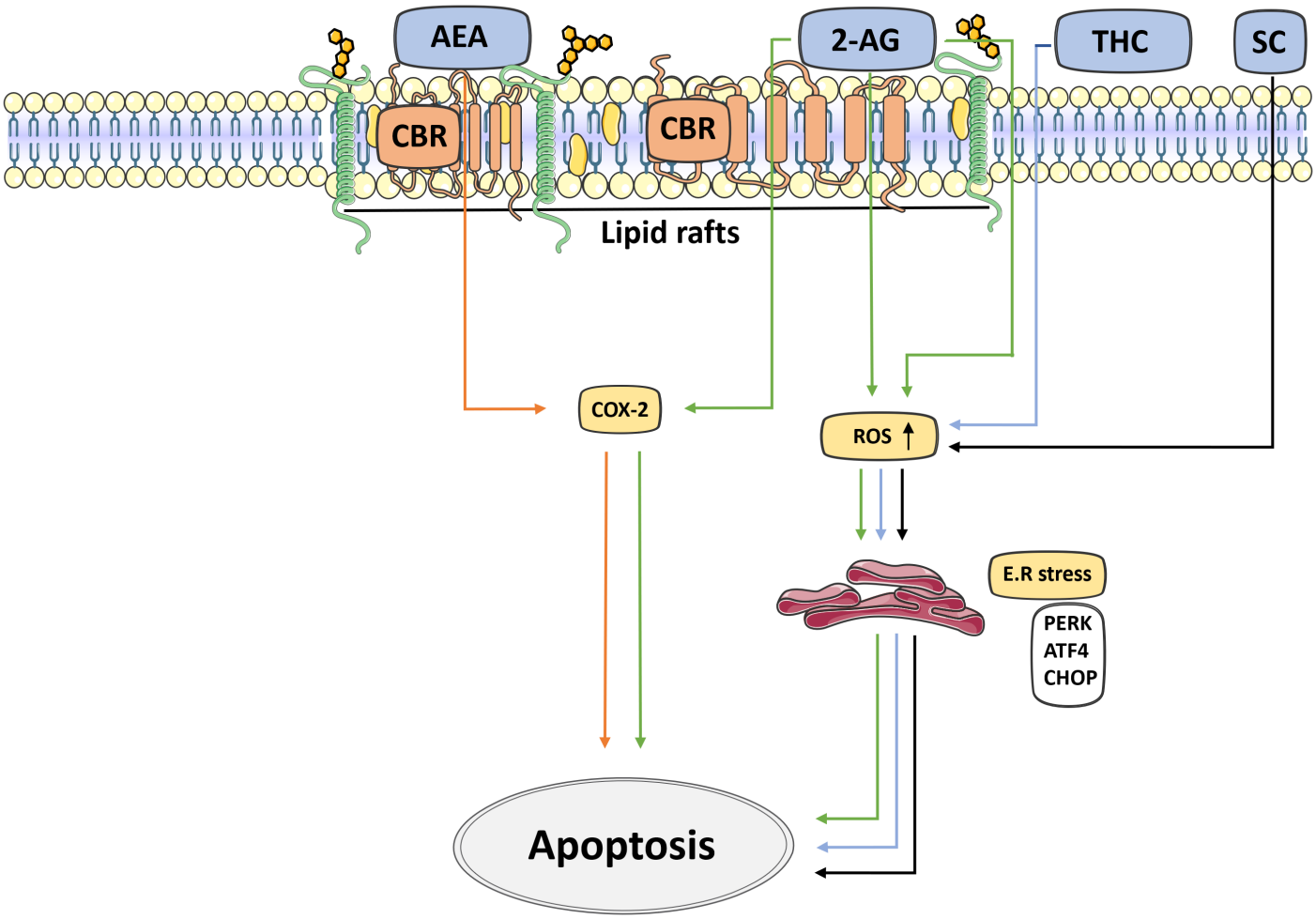

Figure 3

Apoptosis induced by cannabinoids. AEA and 2-AG activate CB1R and COX-2, inducing apoptosis in a manner dependent on the cholesterol-rich lipid rafts in cultured decidual cells. 2-AG causes an increase in ROS, leading to reticulum stress and apoptosis through PERK-ATF4-CHOP in the placenta via CB2R. 2-AG, Δ9-THC and synthetic cannabinoids increased ROS, causing reticulum stress-induced apoptosis in the cytotrophoblastic cell lineage.

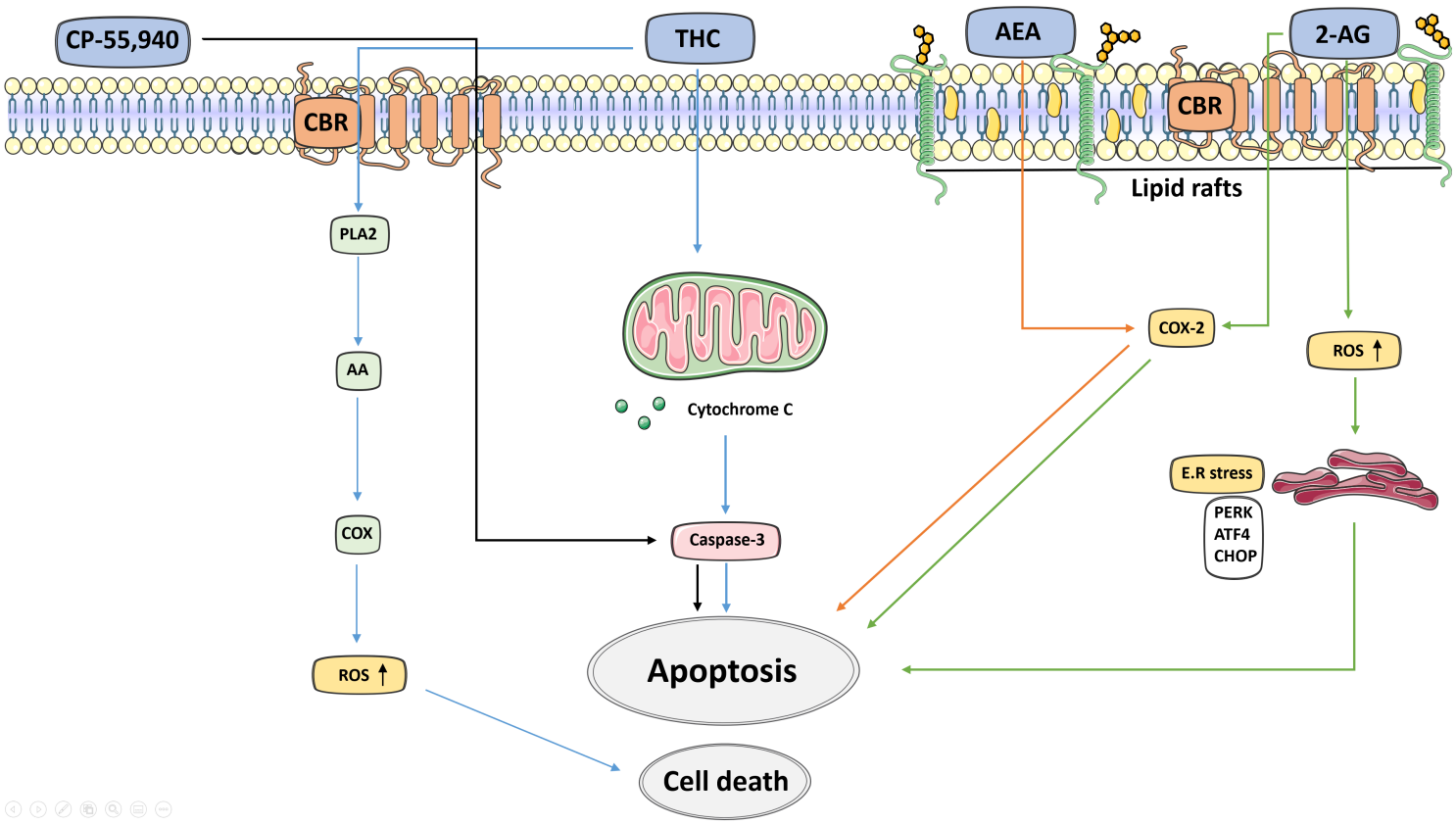

Figure 4

Apoptosis induced by cannabinoids in the CNS. CP-55,940 activates CB1R and causes apoptosis via caspase-3 in forebrain cultures. Δ9-THC, through CB1R, leads to cytochrome c release and caspase-3 activation, inducing apoptosis in cultured cortical neuron cells. Δ9-THC increases AA through PLA2, activating COX-2 and culminating in an increase in ROS, causing hippocampal neural death. P2X7 activation increases the activity of NAPE-PLD and DAGL, leading to an increase in 2-AG and AEA. 2-AG, AEA and WIN activate CB1R and CB2R signaling through P2X7R and cytoplasmic Ca^{2+}, causing an increase in superoxide and cytochrome c release and leading to apoptosis in the retina during development.