The Role of Endocannabinoid Signaling in the Molecular Mechanisms of Neurodegeneration in Alzheimer’s Disease

Gaurav Bedse\textsuperscript{a,b}, Adele Romano\textsuperscript{b}, Angelo M. Lavecchia\textsuperscript{b}, Tommaso Cassano\textsuperscript{a,*} and Silvana Gaetani\textsuperscript{b}

\textsuperscript{a}Department of Biochemical Sciences, Sapienza University of Rome, Rome, Italy
\textsuperscript{b}Department of Physiology and Pharmacology “V. Erspamer”, Sapienza University of Rome, Rome, Italy
\textsuperscript{c}Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

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Abstract. Alzheimer’s disease (AD) is the most common form of progressive neurodegenerative disease characterized by cognitive impairment and mental disorders. The actual cause and cascade of events in the progression of this pathology is not fully determined. AD is multifaceted in nature and is linked to different multiple mechanisms in the brain. This aspect is related to the lack of efficacious therapies that could slow down or hinder the disease onset/progression. The ideal treatment for AD should be able to modulate the disease through multiple mechanisms rather than targeting a single dysregulated pathway. Recently, the endocannabinoid system emerged as novel potential therapeutic target to treat AD. In fact, exogenous and endogenous cannabinoids seem to be able to modulate multiple processes in AD, although the mechanisms that are involved are not fully elucidated. This review provides an update of this area. In this review, we recapitulate the role of endocannabinoid signaling in AD and the probable mechanisms through which modulators of the endocannabinoid system provide their effects, thus highlighting how this target might provide more advantages over other therapeutic targets.

Keywords: 2-AG, Alzheimer’s disease, amyloid-\(\beta\), anandamide, cannabinoids, CB1, CB2, FAAH, MAGL, tau

INTRODUCTION

Alzheimer’s disease (AD) is the most common cause of dementia. About 35.6 million people worldwide are now suffering from AD, and disease prevalence is expected to affect 115 million by 2050 [1]. AD was discovered 100 years ago but the insight into symptoms, etiology, disease progression, pathological mechanism, and treatment has gained a significant progress over last 30 years. Although we have known about this disease for over a century, to date there is no curative treatment available. Three acetylcholinesterase (AChE) inhibitors (donepezil, rivastigmine, and galantamine), and a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, memantine, are the only drugs available and approved by the United States Food and Drug Administration (FDA) for the treatment of AD [2]. The latest (2011) guidance from the National Institute for Health and Clinical Excellence recommends that the three AChE inhibitors are available for managing mild-to-moderate AD, whereas memantine is recommended as an option for treating people

\(\text{*Correspondence to: Tommaso Cassano, Department of Clinical and Experimental Medicine, University of Foggia, Viale Luigi Pinto 1, Foggia - 71100, Italy. Tel.: +39 0881 588042; Fax: +39 0881 188 0432; E-mail: tommaso.cassano@unifg.it.}
Amyloid-Extracellular senile plaques are mainly composed of astrogliosis [15], and microglial cell proliferation [16]. The neurodegeneration in AD is characterized by memory loss, and behavioral changes [11]. Patients suffering from AD exhibit cognitive impairment, memory loss, and behavioral changes [11]. The neurodegeneration in AD is characterized by memory, judgment, and reasoning in the elderly [11]. Neurological disorder that causes deterioration of memory, could be beneficial in AD [4]. However, first glance, it is striking that cannabinoids like delta-9-THC), known to impair produce a wide range of cytokines, such as interleukins [27]. Activated microglia accumulates at the site of Aβ deposition and, as expected, actively engulfs and clears Aβ deposits [28]. Aβ is able to stimulate Src family kinases and Syk tyrosin kinases [29], which further can activate mitogen-activated protein kinase (MAPK) and nuclear factor eB (NF-eB) cascades that are required for proinflammatory cytokine and reactive oxygen species (ROS) production (see Fig. 1) [27]. It has been also reported that Aβ can directly activate MAPK and extracellular signal regulated kinase (ERK) pathways [30]. Transient activation of these signaling pathways after Aβ binding to microtubul results in upregulation of proinflammatory cytokines such as interleukin-1β (IL-1β) and tissue necrosis factor-alpha. The neurodegeneration in AD is characterized by memory loss, and behavioral changes [11]. Patients suffering from AD exhibit cognitive impairment, memory loss, and behavioral changes [11]. The neurodegeneration in AD is characterized by memory, judgment, and reasoning in the elderly [11]. Neurological disorder that causes deterioration of memory, could be beneficial in AD [4]. However, first glance, it is striking that cannabinoids like delta-9-THC), known to impair produce a wide range of cytokines, such as interleukins [27]. Activated microglia accumulates at the site of Aβ deposition and, as expected, actively engulfs and clears Aβ deposits [28]. Aβ is able to stimulate Src family kinases and Syk tyrosin kinases [29], which further can activate mitogen-activated protein kinase (MAPK) and nuclear factor eB (NF-eB) cascades that are required for proinflammatory cytokine and reactive oxygen species (ROS) production (see Fig. 1) [27]. It has been also reported that Aβ can directly activate MAPK and extracellular signal regulated kinase (ERK) pathways [30]. Transient activation of these signaling pathways after Aβ binding to microtubul results in upregulation of proinflammatory cytokines such as interleukin-1β (IL-1β) and tissue necrosis factor-alpha.
Fig. 1. Endocannabinoid signaling and molecular mechanisms of neurodegeneration in AD. Proteolytic cleavage of amyloid-β protein precursor (AβPP) by β- and γ-secretase results in generation of Aβ42 monomers, which under pathological conditions, assembles into oligomers. Aβ42 oligomers activate microglia and astrocytes. Activated microglia produces inflammatory cytokines through nuclear factor κB (NFκB) and mitogen-activated protein kinase (MAPK) pathways. Cytokines released from microglia integrate inflammation process in surrounding astrocytes and neurons through various signaling pathways. Cytokines and Aβ42 through various mechanisms, activate MAPK, NFκB, glycogen synthase kinase-3β (GSK-3β), and caspase-3 pathways. Aβ42 through MAPK and NFκB pathways negatively modulates long-term potentiation by controlling NMDA and mGlu receptor expression, and ultimately causing memory impairment. Moreover, Aβ42 through the activation/expression of kinases, nitric oxide (NO), and caspase-3 increases phosphorylation of tau, which ends in the formation of neurofibrillary tangles (NFTs) in neurons. Under inflammatory conditions both microglia and astrocytes synthesize endocannabinoids (anadamide; AEA and 2-arachidonoylglycerol; 2AG), which through cannabinoid receptors (CB1/CB2) and peroxisome proliferator-activated receptors (PPAR) suppress production of cytokines, iNOS and COX-2 expression. Moreover, AEA augments Notch-1 signaling, which is important in neuronal development, neurogenesis, and neuritic growth. Mitochondrial CB1 receptors inhibit the release of cell apoptotic factors and Ca2+ influx in response to reactive oxygen species. Thus activation of endocannabinoid signaling exerts antioxidant, anti-inflammatory and anti-apoptotic effects. N-acyl-phosphatidyl-ethanolamine; FAE, fatty acid ethanolamides; ERK, extracellular signal regulated kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; AA, arachidonic acid; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; TLR-4, toll-like receptor-4; ADAM, metalloproteinase domain-containing protein; TACE, tumor necrosis factor-α converting enzyme; DSL, Delta/Serrate/LAG-2; NICD, notch intracellular domain; NEXT, notch extracellular truncation; RAGE, receptor for advanced glycation end-products.
The first identified eCB was anandamide (arachidonyl ethanolamide, AEA) [39], which is the derivative of ethanolamine and arachidonic acid (AA). The existence of a second eCB was postulated and soon identified as 2-arachidonylglycerol (2-AG) [40, 41]. 2-AG is an ester derivative of AA and glycerol. The synthesis of AEA and 2-AG is believed to be driven by the cleavage of membrane-associated phospholipids. AEA is synthesized from hydrolysis of N-acyl-phosphatidyl-ethanolamine (NAPE) by phospholipase D (PLD) [42, 43]. 2-AG synthesis derives from the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) and is mediated by the generation of diacylglycerol (DAG), via the actions of either phospholipase C (PLC) or phospholipase D (PLD) [44]. DAG is subsequently converted to 2-AG by DAG lipase [44]. eCBs are produced by a variety of cell types including endothelial cells, adipocytes, glial cells, and macrophages [45–47]. 2-AG is more abundant than AEA in the brain and behaves as a full agonist for CB1 and CB2 receptors, while AEA acts as a partial agonist for CB1 receptors [48]. In addition to CB1 receptors, AEA can also activate peroxisome proliferator-activated-alpha receptors (PPAR-α) and transient receptor potential vanilloid-1 (TRPV1) channels [49]. CB1 receptors are widely expressed throughout the brain [50], predominantly in cerebellum, cortex, hippocampus, and basal ganglia [38]. They are mostly found on axon terminals of a variety of neuronal populations and their activation results in inhibition of adenosyl cyclase activity and calcium influx into the axon terminal; thus, CB1 receptor signaling functions to suppress neurotransmitter release into the synapse [38]. CB2 receptors are also expressed in periphery organs [51]. Following CB1 receptor identification, peripheral CB-receptor was identified and designated as CB2 receptor [52]. CB1 and CB2 receptors are widely distributed in cells and tissues of immune system. Recently, it has been discovered that CB2 is also expressed within the CNS and its expression occurs at various stages of inflammation [53–56]. This expression of CB2 was primarily localized in the microglia and astrocytes [57–59]. Interestingly, CB1 receptor expression can be detected in these cells in CNS only after various insults, whereas it cannot be detected in resting microglia [60]. The CB2 exerts its effects through initiation of phospholipase C (PLC) and inositol 1, 4, 5-trisphosphate (IP3) signaling pathways that result in increased levels of intracellular calcium [59]. There is also evidence on other putative CB-receptor subtypes [61], but no new receptor has been fully characterized or cloned yet. Moreover, it has been proposed that G-protein coupled receptor GPR55 may be a novel cannabinoid receptor [62]. Another suggested putative novel CB-receptor is the TRPV1 receptor, a ligand-gated ion channel [63]. eCBs after their actions are rapidly eliminated by cellular uptake and enzymatic hydrolysis. After cellular re-uptake AEA is metabolized by the fatty acid amide hydrolase (FAAH) [64] expressed mostly by postsynaptic neurons. FAAH metabolizes also other N-acyl ethanolamines, like palmitoylethanolamide (PEA) and oleoylethanolamide.
ENDOCANNABINOID SIGNALING IN ALZHEIMER’S DISEASE

Multiple data are available showing that the eCB system is implicated in AD progression. Cortical and hippocampus, key structures for learning and memory functions, are the two brain regions that are affected by AD pathology [72], and they express high levels of CB1 receptors as well as other components of the eCB system [73]. Evidence suggests that microglia and astrocytes also express the enzymes involved in the synthesis and degradation of the eCBs and that the activation of cannabinoid receptors expressed by activated microglia controls immune-related function [59]. Moreover, eCBs are known to exert anti-inflammatory, antioxidant, and neuroprotective effects [7, 74–77]. Therefore, it is not surprising that eCB signaling plays a crucial role in AD. Table 1 compiles all reports addressing the expression levels of eCB signaling components in AD in humans as well as in in vitro and in vivo preclinical models. The major implications of dysregulated eCB signaling in AD are briefly discussed below.

The relationship of CB1 receptors and AD is sparse and often contradictory in the literature. Westlake and colleagues evaluated the CB1 mRNA expression and 

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^{[3]H}CP-55,940/(CB1 and CB2 agonist) binding density in postmortem AD human brains [78]. \]

 binding was reduced but no alterations in CB1 expression levels were observed in AD brains compared to aged-matched controls. Though, \([^{3}H]CP-55,940\) binding was reduced, it was not selectively associated with the AD pathology. In accordance with this report, other research groups found that CB1 receptor levels were unaltered in patients suffering from AD [79–81]. In contrast, significant decrease in CB1 receptor expression has been reported in the cortex of AD patients [82, 83]. CB1 expression was greatly reduced and CB1 protein nitration was enhanced in the areas of microglial infiltration in AD brains [82]. However, reduced CB1 levels were correlated to hypophagia but not with any AD molecular marker or cognitive status [83]. Furthermore, CB1 receptor selective radioligand study revealed that CB1 receptor density increases in early AD and decreases during later disease stages [84]. In line with these results, two recent papers by our group [85] and by Kalifa and his colleagues [86] reported a decrease in CB1 protein expression in transgenic mice models of AD. However, we found that in aged triple transgenic mice of AD (3×Tg-AD) CB1 mRNA was significantly increased in limbic brain areas. Though we did not find a direct correlation between CB1 mRNA and CB1 protein, an inverse correlation between CB1 protein levels and Aβ protein were observed in hippocampus and basolateral amygdala [85]. The reduced CB1 expression in AβPPswapoPS1ΔE9 mice was associated with astrogial proliferation and elevated expression of cytokines, iNOS and TNF-α [86]. Similarly, pre-treatment with Aβ42 in rats and C6 rat astrogliaoma cells can cause a down-regulation of CB1 receptor [87]. Furthermore, Ahmad and colleagues investigated the availability of CB1 receptor in AD patients by positron emission tomography. This study neither found any difference in CB1 receptor availability between AD and healthy volunteers nor found a correlation between CB1 receptor and Aβ deposition [88]. Even though CB1 receptors were unchanged, it was proposed that the coupling between receptor and G protein could underlie the reduced signaling of CB1 receptor [89]. A recent study further showed that CB1 receptor activity depends on the AD stages. CB1 activity was found higher at earlier AD stages in limited hippocampal areas and internal layers of frontal cortex, but a decrease was observed at the advanced stages [90]. The
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Tissue</th>
<th>Component of eCB system</th>
<th>Observation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human AD patient</td>
<td>Cortex, Hippocampus, Striatum, Anterior cingulate gyrus, Caudate nucleus, Basal ganglia, Brainstem</td>
<td>CB1 protein and binding</td>
<td>Unchanged</td>
<td>[79–81, 88]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Hippocampus, Neocortex, Basal ganglia, Brainstem</td>
<td>CB1 mRNA CB1 binding</td>
<td>CB1 mRNA - Unchanged</td>
<td>[78]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Cortex</td>
<td>CB1 protein</td>
<td>Unchanged</td>
<td>[82, 83]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Blood</td>
<td>CB1 mRNA and protein</td>
<td>CB1 mRNA-altered</td>
<td>[85]</td>
</tr>
<tr>
<td>3 × Tg-AD mice</td>
<td>Hippocampus, BLA, Prefrontal cortex</td>
<td>CB1 mRNA and protein</td>
<td>CB1 protein-reduced in dorsal hippocampus and BLA</td>
<td>[191]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Prefrontal cortex</td>
<td>CB1 binding</td>
<td>CB1 density increases in early AD followed by decreases during later disease stages</td>
<td>[84]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Prefrontal cortex, Hippocampus</td>
<td>CB1 receptor-dependent Gi protein activation</td>
<td>CB1 activity increased at earlier AD stages and decreased at advance stages</td>
<td>[90]</td>
</tr>
<tr>
<td>AβPP+PSE/ΔE9 mice</td>
<td>Hippocampus</td>
<td>CB1 protein</td>
<td>Decreased</td>
<td>[86]</td>
</tr>
<tr>
<td>AβPP+PSE/ΔE9 mice</td>
<td>Hippocampus</td>
<td>CB1 receptor-dependent Gi protein activation</td>
<td>CB1 and CB1 mRNA-protein</td>
<td>[87]</td>
</tr>
<tr>
<td>Rat (Aβ42 insult)</td>
<td>Brain/Cells</td>
<td>CB1 protein</td>
<td>CB1- Decreased</td>
<td>[87]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Cortex, Hippocampus, Blood</td>
<td>CB2 protein and mRNA</td>
<td>Increased</td>
<td>[79, 82, 91, 92, 97]</td>
</tr>
<tr>
<td>Human DS patient</td>
<td>Prefrontal cortex, Cortex</td>
<td>CB1 protein and FAAH protein</td>
<td>CB1 binding</td>
<td>Increased</td>
</tr>
<tr>
<td>AβPP+PSE/ΔE9 mice</td>
<td>Prefrontal cortex, Hippocampus</td>
<td>CB1 receptor-dependent Gi protein activation</td>
<td>CB1 activity increased</td>
<td>[96]</td>
</tr>
<tr>
<td>AβPP/PSE/Neuro-2a cells</td>
<td>Neuro-2a cells</td>
<td>FAAH</td>
<td>Increased activity and expression</td>
<td>[93]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Prefrontal cortex, Hippocampus</td>
<td>FAAH</td>
<td>Increased</td>
<td>[93]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Prefrontal cortex</td>
<td>FAAH protein, mRNA and activity</td>
<td>Increased</td>
<td>[79, 192]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Cortex, blood</td>
<td>FAAH protein, mRNA and activity</td>
<td>Increased</td>
<td>[79, 192]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Cortex</td>
<td>AEA and NaPE</td>
<td>Decreased</td>
<td>[93]</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Plasma</td>
<td>AEA and 2-AG</td>
<td>Unchanged</td>
<td>[98]</td>
</tr>
<tr>
<td>PS1/AβPP mice</td>
<td>Whole brain</td>
<td>AEA and 2-AG</td>
<td>Increased</td>
<td>[99]</td>
</tr>
<tr>
<td>Rats (Aβ42 insult)</td>
<td>Ch glia cells, Hippocampus, Striatum</td>
<td>AEA and 2-AG</td>
<td>2-AG-Increased</td>
<td>[87, 101]</td>
</tr>
<tr>
<td>AβPP+PSE/ΔE9 mice</td>
<td>Frontal cortex, Hippocampus, Striatum</td>
<td>AEA, 2-AG, PEA and OEA</td>
<td>AEA-decreased</td>
<td>Decreased only in striatum</td>
</tr>
<tr>
<td>Human AD patient</td>
<td>Hippocampus</td>
<td>DAGL, MAGL, ABHD6</td>
<td>DAGL, MAGL-decreased ABHD6,abolished</td>
<td>[80]</td>
</tr>
</tbody>
</table>

CB1and CB2, cannabinoid receptors; BLA, basolateral amygdala; DS, Down’s syndrome; FAAH, fatty acid amide hydrolase; NaPE, 2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine-N-arachidonoyl; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; DAGL, diacetyl glycerol lipase; MAGL, monoacylglycerol lipase; ABHD6, serine hydrolase a/j hydrolyase 6.

increased CB1 receptor activity during the initial stages of AD might indicate neuroprotective action medicated by CB1 receptors and FAAH increased CB1 receptor activity. In fact, postmortem brains from patients with AD revealed that CB1 receptors and FAAH are selectively overexpressed in cells that are associated to Aβ-enriched neuritic plaques [79, 80, 82, 83, 91, 92]. The hydrolytic activity of FAAH is well documented in the literature.
enhanced in Aβ42 plaques and surrounding areas [79, 93]. Increased FAAH activity may contribute to inflammatory processes by increasing AA (precursor for proinflammatory molecules) through increased AEA metabolism in astrocyte cells surrounding plaques. Moreover, FAAH is selectively overexpressed in reactive astrocytes and CB2 receptors are overexpressed in activated microglial cells in AD [79, 94, 95]. Similarly, in Down’s syndrome, characterized by Aβ deposition, increased FAAH activity and CB2 expression have been observed [95]. Moreover, increased levels of CB2 receptors were positively correlated with Aβ42 and senile plaque score [83]. Apart from human studies, transgenic model of AD has also revealed overexpression of CB2 receptors in brain areas affected by the AD-pathology [96]. Increased CB2 mRNA in peripheral blood has been suggested as a peripheral biomarker for the early diagnosis of AD [97]. Pretreatment with AEA, to rats and C6 rat astroglia cells also increases CB2 receptor expression [87].

Since AEA and, to a lesser extent, 2-AG are the substrates of FAAH, reduction in AEA and/or 2-AG can be expected in brain areas severely affected by AD pathology. In line with this, Jung and colleagues reported that AEA and its precursor 1-steinoyl, 2-docosahexaenoyl-sn-glycero-phosphoethanolamine-N-arachidonoyl (NarPE) levels, but not 2-AG, were significantly reduced in cortex of AD patients [93]. However, AEA and 2-AG plasma levels were unchanged in AD patients compared to healthy volunteers [98]. Moreover, AEA and NarPE levels in cortex were positively correlated to cognitive impairment and inversely correlated to Aβ load; however, no correlation was found with plasma eCBs and cognitive performance [93, 98]. Conversely, AEA and 2-AG levels were found to be increased in brains of the PS1/APP transgenic male of AD [99]. Mulder and colleagues found that 2-AG signaling is altered in postmortem AD brains. The expression of 2-AG degrading enzymes, MAGL and ABHD6, was differentially altered in hippocampal neurons. ABHD6 expression was completely abolished and MAGL expression was lowered in NFT-bearing pyramidal neurons. This study demonstrated that AD progression slows down the termination of 2-AG signaling and that could contribute to synapse silencing particularly around senile plaques [80]. Apart from postmortem analyses and transgenic models of AD, studies on animal models of AD induced by acute administration of Aβ42 have also shown the increase of DAG lipase and 2-AG levels [87, 101].

**BENEFICIAL EFFECTS OF CANNABINOIDS IN TREATMENT OF ALZHEIMER’S DISEASE**

Increasing evidence suggests that the eCB system could be a potential target for the treatment of AD. During the last decade, an ample number of interesting studies allowed for a new perspective into the prevention and/or treatment of AD focusing on the eCB system (for review, see [5, 10–74, 76, 102–104]). Cannabinoids could exert neuroprotective, antioxidant, anti-apoptosis, and anti-inflammatory effects [77]. Cannabinoids play a neuroprotective role, through the CB2 receptor activation, by preventing excitotoxicity, calcium efflux, and inflammation as well as by modulating other signaling pathways [105]. Most of the initial reports on the effects of cannabinoids in AD were investigated in in vitro models of Aβ-induced neuronal toxicity. Later, these investigations were extended to animal models of Aβ-induced toxicity and to transgenic murine models expressing plaques and/or tangles pathology. Table 2 compiles the in vitro and in vivo experimental evidence of beneficial effects of cannabinoids in AD treatment. Figure 1 summarizes the probable molecular and cellular mechanisms underlying these beneficial effects. In the following section the effects of cannabinoids on various pathological processes of AD will be discussed.

*Aβ* generation and clearance

Microglia plays an important role in phagocytosis of Aβ, and there is an inverse relationship between cytokine production and Aβ clearance [26, 106]. CB2 activation is known to reduce microglia activity and inflammatory cytokines productions [107]. So it can be hypothesized that CB2 agonist could lower Aβ plaque load by increasing Aβ clearance. In line with this hypothesis, it has been shown that in vitro activation of CB2 receptor facilitates the removal of native Aβ from human frozen tissue sections as well as the removal of synthetic pathogenic peptide by a human macrophage cell line [108]. Moreover, a CB2 agonist was able to induce a prompt Aβ clearance in Aβ-induced animal model of AD [109]. The mechanism underlying CB2 mediated decrease in Aβ plaque load is not clear yet. However, it was suggested that it might be link to a lower the production of inflammatory cytokines and increase of Aβ phagocytosis that might decrease Aβ
Table 2
Beneficial effects of modulators of the endocannabinoid system and their molecular mechanisms in AD

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Treatment</th>
<th>Effects and mechanisms involved</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ntera 2/D1 neurons (Aβ insult)</td>
<td>AEA</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
</tr>
<tr>
<td>Wistar rats (Aβ insult)</td>
<td>VDM-11</td>
<td>MAPK pathway activation</td>
<td>[101]</td>
</tr>
<tr>
<td>PC12 cells</td>
<td>AEA</td>
<td>↑ cell viability</td>
<td>[150]</td>
</tr>
<tr>
<td>SH-SY5Y cells (Aβ and peroxide insult)</td>
<td>Noladin ether</td>
<td>MAPK pathway activation</td>
<td>Improved memory retention</td>
</tr>
<tr>
<td>Cell lines</td>
<td>VDM-11</td>
<td>Improved memory retention</td>
<td>[112]</td>
</tr>
<tr>
<td>Primary hippocampal neurons (Aβ insult)</td>
<td>VDM-11</td>
<td>↑ cell viability</td>
<td>Improved memory retention</td>
</tr>
<tr>
<td>PC12 cells</td>
<td>AEA</td>
<td>MAPK pathway activation</td>
<td>[150]</td>
</tr>
<tr>
<td>SH-SY5Y cells (Aβ and peroxide insult)</td>
<td>CB1 mediated effect</td>
<td>↑ cell viability</td>
<td>Improved memory retention</td>
</tr>
<tr>
<td>Vitro model of the BBB</td>
<td>2-AG</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
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<tr>
<td>2-AG</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
<td></td>
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<tr>
<td>2-AG</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
<td></td>
</tr>
<tr>
<td>Primary hippocampal neurons (Aβ insult)</td>
<td>JZL185</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
</tr>
<tr>
<td>2-AG</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
<td></td>
</tr>
<tr>
<td>Primary hippocampal neurons (Aβ insult)</td>
<td>JZL184</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
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<tr>
<td>JZL185</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
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<tr>
<td>Primary hippocampal neurons (Aβ insult)</td>
<td>JZL184</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
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<tr>
<td>JZL185</td>
<td>↓ Aβ toxicity</td>
<td>[149]</td>
<td></td>
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<tr>
<td>Mouse astrocytes (Aβ treatment)</td>
<td>AEA, PEA and OEA</td>
<td>↓ inflammation</td>
<td>[137]</td>
</tr>
<tr>
<td>eCB degradation enzyme inhibitors</td>
<td>AEA, 2-AG, URB597</td>
<td>↓ inflammation</td>
<td>[137]</td>
</tr>
<tr>
<td>Primary cortical neurons (Aβ treatment)</td>
<td>AEA, PEA, OEA</td>
<td>↓ inflammation</td>
<td>[137]</td>
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<tr>
<td>AβPP/PS1 AD mice</td>
<td>Genetic/pharmacological inactivation of MAGL</td>
<td>↓ inflammation</td>
<td>[99]</td>
</tr>
<tr>
<td>AβPP/PS1 AD mice</td>
<td>↓ inflammation</td>
<td>[99]</td>
<td></td>
</tr>
<tr>
<td>5×FAD AβPP transgenic mice</td>
<td>JZL184</td>
<td>↓ inflammation</td>
<td>[99]</td>
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<td>Cannabinoid agonists</td>
<td>Microglial cells (Aβ insult)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
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<tr>
<td>Human fetal astrocytes (IL-1β insult)</td>
<td>WIN55,212-2 (mixed CB1 / CB2 agonist)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
<tr>
<td>C6 rat glioma cells (Aβ insult)</td>
<td>WIN55,212-2 (mixed CB1 / CB2 agonist)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
<tr>
<td>SD rats brain slices</td>
<td>WIN55,212-2 (mixed CB1 / CB2 agonist)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
<tr>
<td>Wistar rats (Aβ insult)</td>
<td>WIN55,212-2 (mixed CB1 / CB2 agonist)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
<tr>
<td>Rats (Aβ insult)</td>
<td>WIN55,212-2 (mixed CB1 / CB2 agonist)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
<tr>
<td>Adipocytes (mixed CB1 / CB2 agonist)</td>
<td>HU210 (mixed CB1 / CB2 agonist)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
<tr>
<td>Microglial cells (Aβ insult)</td>
<td>JWH-015 (CB2 agonist)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
<tr>
<td>Human brain microvascular endothelial cells, mice</td>
<td>JWH133 (CB2 agonist)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
<tr>
<td>Rats (Aβ insult)</td>
<td>JWH133 (CB2 agonist)</td>
<td>↓ microglia-mediated neurotoxicity</td>
<td>[82]</td>
</tr>
</tbody>
</table>

**Microglial cells (Aβ insult):**
- JWH-015 (CB2 agonist)
- JWH133 (CB2 agonist)
- JZL184 (CB1 agonist)
- HU210 (mixed CB1 / CB2 agonist)
- WIN55,212-2 (mixed CB1 / CB2 agonist)

**Human brain microvascular endothelial cells, mice:**
- JWH133 (CB2 agonist)

**Rats (Aβ insult):**
- JWH133 (CB2 agonist)
- HU210 (mixed CB1 / CB2 agonist)
- WIN55,212-2 (mixed CB1 / CB2 agonist)
- JZL184 (CB1 agonist)
- JWH133 (CB2 agonist)

**Adipocytes (mixed CB1 / CB2 agonist):**
- HU210 (mixed CB1 / CB2 agonist)

**Microglial cells (Aβ insult):**
- JWH-015 (CB2 agonist)
- JWH133 (CB2 agonist)
- JZL184 (CB1 agonist)
- HU210 (mixed CB1 / CB2 agonist)
- WIN55,212-2 (mixed CB1 / CB2 agonist)
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Treatment</th>
<th>Effects and mechanism involved</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tg AβPP mice</td>
<td>FSH-133 (CB1 agonist)</td>
<td>Improves cognitive performance ↓ Br-1, COX-2, TNF-α</td>
<td>[142]</td>
</tr>
<tr>
<td>Pharmacological or genetic inhibition of cannabinoid receptors swiss mice (AβPP−/− mice)</td>
<td>Rimonabant (CB 1 antagonist)</td>
<td>Improves Aβ-induced amnesia ↓ tau hyperphosphorylation</td>
<td>[173]</td>
</tr>
<tr>
<td>AβPP/CB1−/− mice</td>
<td>Membrane deficits ↑ Aβ plaque ↓ microglia activity ↓ soluble tau</td>
<td>[110]</td>
<td></td>
</tr>
<tr>
<td>AβPP/CB2−/− mice</td>
<td>Membrane deficits ↑ Aβ plaque ↓ microglia activity ↓ soluble tau</td>
<td>[110]</td>
<td></td>
</tr>
<tr>
<td>Phytocannabinoids Rat cortical neuron culture (glutamate insult)</td>
<td>Cannabidiol Δ9-THC</td>
<td>glutamate toxicity ↓ ATP-induced Ca2+↑</td>
<td>[158]</td>
</tr>
<tr>
<td>microglial cells C57/B6 Mice (Aβ42 insult)</td>
<td>Cannabidiol WIN 55,212-2 FSH-133</td>
<td>ATP-induced Ca2+↑ ↓ microglia migration ↑ NO, TNF-α, IL-6 ↑ oxidative impairment</td>
<td>[131]</td>
</tr>
<tr>
<td>C57BL/6J mice (Aβ42 insult)</td>
<td>cannabidiol</td>
<td>GFAP ↓ GSK3β and p-GSK3β↑</td>
<td>[132]</td>
</tr>
<tr>
<td>AChE from Electrophorus electricus</td>
<td>cannabinoids</td>
<td>↓ AChE-induced Aβ aggregation</td>
<td>[175]</td>
</tr>
<tr>
<td>N2a/AβPP/Cre cells</td>
<td>cannabinoids</td>
<td>Aβ fibrils ↓ p-GSK3β↑↑</td>
<td>[109]</td>
</tr>
<tr>
<td>PC12 neuronal cells (Aβ42 insult)</td>
<td>cannabinoids</td>
<td>↓ NO, TNF-α, IL-6, S100B ↓ reactive gliosis ↑ NF-κB↑↑</td>
<td>[133]</td>
</tr>
<tr>
<td>PC12 cells (Aβ42 insult)</td>
<td>cannabinoids</td>
<td>neuronal cell viability ↑ neuroprotective actions</td>
<td>[133]</td>
</tr>
<tr>
<td>Neuroblastoma cells (Aβ42 insult)</td>
<td>Aβ fibrils ↓ p38 MAP kinase ↑ PPAR-γ</td>
<td>Mediated through PPAR-γ</td>
<td>[109]</td>
</tr>
<tr>
<td>PC12 cells (Aβ insult)</td>
<td>Aβ fibrils ↓ p38 MAP kinase ↑ PPAR-γ</td>
<td>Mediated through PPAR-γ</td>
<td>[109]</td>
</tr>
<tr>
<td>AβPP/PS1 mice</td>
<td>Cannabidiol + Δ9-THC</td>
<td>Neuronal plasticity, neuroprotection, anti-inflammatory actions</td>
<td>[171]</td>
</tr>
</tbody>
</table>

Plaque load [107]. The role of CB2 receptors in lowering Aβ plaques was further confirmed by a study where CB2 receptors were deleted in AβPP mutant mice (PDGFβ-AβPPswInd). Results from this study revealed that soluble Aβ and plaque deposition were significantly increased in AβPP/CB2−/− mice compared to AβPP/CB2+/+ mice [110]. The exact role of CB1 receptor is not yet clear in same context. Effect of cannabinoid treatment on Aβ fibril and aggregate formation was recently...
inhibition of NO-dependent tau hyperphosphorylation
2´-chloroethylamide (ACEA), a selective CB1 agonist, down regulates iNOS protein expression and NO production in astrocytes, and that leads to a significant inhibition of NO-dependent tau hyperphosphorylation in neurons [121]. In another report [122], it has been demonstrated that cannabidiol (a non psychoactive component of marijuana) inhibits hyperphosphorylation of tau protein in Aβ-stimulated neural cells. The effect of cannabidiol was mediated through the Wnt/β-catenin pathway [132]. Wnt activation leads to inhibition of GSK-3β, which is also known as tau protein kinase, responsible for a massive tau protein hyperphosphorylation and relative NFT formation observed in brains of AD patients [123]. A recent report also demonstrated that Δ⁹-THC treatment inhibits activation of GSK-3β in N2a-variant APP mice [124].

**Neuroinflammation**

Besides plaques and NFTs, neuroinflammation plays a major role in neurodegeneration and activation of various apoptosis pathways. The notion that Aβ is a pathological molecule is slowly changing and it seems that it represents a cellular adaptive strategy to oxidative stress [125]. Aβ is a proinflammatory molecule, which can induce its own production by increasing the expression of its synthesizing enzymes such as β-secretase (BACE1) and through various inflammatory pathways [125]. In particular, it has been recognized that Aβ is able to initiate an inflammatory response, which in turn activates microglia and recruits astrocytes, and therefore the release of inflammatory mediators (IL-1β, TNF-α, and IL-6), reactive oxygen species (NO), and neurotoxic products that have been involved in neuronal and synaptic damage [31]. Neuroprotective effects of eCBs against brain injury and inflammation is associated with reduction of cytokines, ROS, and prostaglandins [126–128]. eCB modulators can reduce neuroinflammation in AD by inhibiting glial cell activation and generation of proinflammatory precursor molecules.

**Regulation of glial cell activity**

As discussed earlier in this review, CB2 and FAAH expression is upregulated in microglia and astrocytes, respectively, in surrounding areas of neuritic plaques in AD brains. This notion suggests that both microglia and astrocytes play an important role in eCB signaling in AD pathology. It seems that upregulation of CB2 receptor in AD is a defensive mechanism to limit inflammation and to clear plaques from the affected brain region [79, 110, 129]. CB2 receptors are coupled to Gi/o inhibitory proteins so that their activation is associated with inhibition of adenyl cyclase and the cAMP/protein kinases A (PKA) dependent pathway [130]. CB2 receptor activation could provide...
beneficial effects at various levels. In particular, CB2 activation could 1) suppress activation of microglia, 2) reduce production of inflammatory molecules like IL-1β, IL-6, TNF-α, NO, etc., 3) enhance microglial proliferation, and 4) enhance microglial phagocytic activity [59, 82, 107, 108, 131].

The effects of non selective cannabinoid agonists on microglial activation were demonstrated by Ramirez and colleagues [82]. In their study authors investigated the effects of non selective cannabinoids and selective CB2 agonist in Aβ-induced microglial cells [82]. As expected, Aβ peptide activated microglial cells with increased mitochondrial activity, TNF-α release, and cellular morphological changes. Cannabinoid treatment prevented the enhancement of TNF-α release and counteracted Aβ-mediated activation of microglia. Furthermore, mechanistic insight of beneficial effects provided by CB2 receptor stimulation in AD was demonstrated. Stimulation of CB2 receptor significantly attenuated CD40-mediated inhibition of microglial phagocytosis of Aβ42 peptide [107].

Cannabidiol dose dependently reduced Aβ-induced neuroinflammation by suppressing microglial activation, IL-1β and iNOS expression [132].

It has been also shown that cannabinoid treatment, in activated astrocytes, inhibits synthesis of inflammatory chemokines and NO release [133]. Win55,212-2, an agonist of CB1 and CB2 receptors, inhibited inducible NO synthase (iNOS) and corresponding NO production in astrocytes activated by IL-1β [134]. Win55,212-2 treatment also inhibited production of chemokines (CXCL10, CCL2, and CCL5) and TNF-α. Both selective CB1 and CB2 antagonists partially blocked these effects suggesting the involvement of both receptors [134]. Cannabidiol markedly down-regulates, in a PPAR-γ dependent manner, Aβ-induced reactive gliosis by reducing pro-inflammatory molecules and cytokine release [133]. PPAR-γ activation could inhibit NFκB pathway, which is involved in the synthesis of inflammatory cytokines [135, 136]. In another report, different N-acylethanolamines (AEA, PEA, and OEA) were able to exert anti-inflammatory effects in Aβ-activated murine astrocytes [137]. Previous studies have shown that N-acylethanolamines activate anti-inflammatory nuclear receptor PPAR-α that causes formation of a multiprotein complex along with variable set of protein co-activators [138]. With this multiprotein complex, PPAR-α binds to responsive elements on DNA and enhances the transcription of various anti-inflammatory proteins, such as inhibitor of κB-α (IκB-α), that suppress the gene expression of pro-inflammatory components, such as cytokines (TNF-α, IL-1β) including iNOS and COX-2 (see Fig. 1) [138, 139]. Anti-inflammatory effects of cannabinoids have been also demonstrated in Aβ-induced in vivo AD models [129, 140] and transgenic mice models of AD [141].

Regulation of pro-inflammatory precursors

Phospholipase A2 (PLA2) enzymes are considered the primary source of AA for COX-mediated biosynthesis of prostaglandins [142]. Recently, Nomura and colleagues [143] have shown that MAGL-mediated hydrolysis of 2-AG can act as a distinct pathway to generate AA in the brain [143]. In line with this report, two independent research teams [99, 115] reported that the inactivation of MAGL reduced neuroinflammation, neurodegeneration, and the production and accumulation of Aβ plaques in the transgenic mice of AD. These effects were not mediated by CB1 and/or CB2 receptors but were caused by reduced production of AA [99, 146]. The inhibition of MAGL also improved the neuronal plasticity and learning and memory deficits [99, 113]. Inactivation of MAGL for eight weeks was sufficient to decrease production and deposition of Aβ plaques and the function of BACE1, the enzyme involved in making toxic Aβ in the brain (Fig. 2) [115]. These results suggest that MAGL contributes to the cause and development of AD and that the inhibition of MAGL might represent a promising potential therapeutic target.

MGL inhibition can cause an elevation of 2-AG endogenous levels. In turn, 2-AG by activating CB1 receptor is able to suppress COX-2 elevation in response to inflammatory insult like lipopolysaccharide [144]. Furthermore, it was revealed that the neuroprotective effects of 2-AG were mediated by CB1 but not by CB2 or TRPV1 receptors [145]. CB1 receptor activation by 2-AG suppresses phosphorylation of ERK1/2/p38MAPK/NfκB in neurons, which further suppresses COX-2 expression (Fig. 2) [144, 145]. COX-2 plays an important role in production of prostaglandins, which are crucial in neuroinflammation [142]. Further research in this field revealed that PPAR-γ mediates 2-AG-induced inhibition of NFκB phosphorylation and COX-2 expression in response to pro-inflammatory IL-1β. Moreover, 2-AG is able to restore IL-1β-induced reduction of PPAR-γ expression in CB1 dependent mechanism [146]. Inflammation activates the transcription factor NFκB, for which β-secretase (BACE1) promoter harbors a highly conserved binding site that is functional [125]. Thus NFκB activates BACE1 promoter, expression, and phosphorylation.
Fig. 2. Modulation of 2-AG signaling provides anti-inflammatory effects in AD. Through a CB1-dependent mechanism, 2-AG increases PPAR-\(\gamma\) expression, which is suppressed by A\(\beta\) in AD. 2-AG directly, through CB1 and PPAR-\(\gamma\) receptors, inhibits the expression of COX-2 and the synthesis of inflammatory cytokines. COX-2 plays a major role in the synthesis of proinflammatory prostaglandins from arachidonic acid (AA), which is a degradation product of 2-AG. Proinflammatory prostaglandins can increase neuroinflammation as well as the expression and activity of \(\beta\)- and \(\gamma\)-secretase resulting in increased A\(\beta\) production. Inflammation activates the transcription factor NF\(\kappa\)B, for which \(\beta\)-secretase (BACE1) promoter harbors a highly conserved binding site that is inducible. Thus, NF\(\kappa\)B activates BACE1 promoter, expression, and enzymatic activity leading to increased A\(\beta\) production. Prostaglandin PGE2 stimulates the generation of A\(\beta\) through both EP2 and EP4 receptors (PGE2 receptors). Activation of the EP4 receptor stimulates A\(\beta\) production through endocytosis and the activation of \(\gamma\)-secretase. The inhibition of prostaglandin synthesis by MAGL inhibitors could suppress all these mechanisms.

Neurodegeneration

A\(\beta\) has been shown to induce cell apoptosis in neuronal cells through a variety of mechanisms that include activation of caspase-3, lysosomal cathepsins, and lysosomal membrane permeabilization [17, 118]. Cannabinoids at physiological concentrations increase lysosomal stability and integrity [148]. Noonan and colleagues showed that eCBs can stabilize lysosomes against A\(\beta\) permeabilization and can increase cell survival. eCBs prevented upregulation of tumor suppressor protein, p53, and reduced its interaction with lysosomal membrane [148]. Moreover, 2-AG and AEA prevented A\(\beta\)-induced increase in DNA fragmentation and caspase-3 activation [101]. Acute in vivo administration of A\(\beta\) increases 2-AG release in the brain suggesting that endogenous 2-AG plays an important role in protecting neurons from A\(\beta\)-induced toxicity [101]. Milton and colleagues [149] showed the neuroprotective effects of eCBs (AEA and nodaline ether) on A\(\beta\)-induced neurotoxicity. These effects were mediated by CB1 receptors and the MAPK pathway activation as suggested by the finding that CB1 antagonist and MAPK inhibitor blocked their neuroprotective effects. Another study confirmed the neuroprotective effect of AEA on A\(\beta\)-evoked neurotoxicity via a pathway unrelated to CB1 and CB2 [150]. In fact, selective CB1 and CB2 agonists were unable to protect neurons against A\(\beta\)-challenge [150]. Further research revealed that increasing endogenous levels of 2-AG by MAGL inhibitor was able to protect hippocampal neurons from A\(\beta\)-induced neurodegeneration and apoptosis [145]. Active caspase-3 levels are increased in AD [118]. CB1 agonist was also able to inhibit A\(\beta\)-induced activation of caspase-3 [145, 151]. CB1 knock-out studies indicated that lack of CB1 is associated with increased enzymatic activity leading to increased A\(\beta\) production.
caspase activation and greater loss and/or alterations of myelin and axonal/neuronal proteins [152].

Oxidative damage and mitochondrial dysfunction

Enhanced oxidative stress in brain generally correlates with cognitive decline and with enhanced risk for development of neurodegenerative diseases. Among the different pro-inflammatory proteins produced in response to Aβ-induced oxidative stress, iNOS and its enzymatic product NO [105, 153] are considered the most important neurotoxic effectors during AD. In particular, methionine-35 of Aβ is critical for oxidative stress (for more details, see [154]). Nvx-B, a redox-sensitive transcription factor that is activated by a family of stress activated kinases (SAPK) including p38 MAP kinase [122], regulates the expression of different genes involved in cell differentiation, proliferation, and apoptosis, as well as in oxidative, inflammatory, and immune response [155]. As it is well known, NvxB activation is of primarily importance to induce iNOS protein transcription [156] both in Aβ-stimulated neuronal cells [156] and in postmortem AD brains [157]. It is well known that phytocannabinoids have anti-oxidant properties [158]. Cannabidiol is a well studied cannabinoid in this context. It has been shown that cannabidiol significantly decreases glutamate toxicity, Ca2+ toxicity, iNOS expression, and NO production [131, 158]. Cannabidiol mediates these effects through inhibition of p38 MAPK and NvxB pathways probably through involvement of the PPAR-γ receptor [132, 133, 135]. Moreover, CB1 agonists were also shown to decrease iNOS and NO production [121, 131]. In another study, cannabidiol treatment significantly decreased ROS, lipid peroxidation, caspase-3 levels, DNA fragmentation and intracellular calcium [156]. CB1 receptor activity on hippocampal GABAergic neurons protects against age-dependent cognitive decline by reducing pyramidal cell degeneration and neuroinflammation [169]. The same authors suggested that CB1 receptor activity on hippocampal GABAergic neurons protects against age-dependent cognitive decline by reducing pyramidal cell degeneration and neuroinflammation [169].

Moreover, the consequences of CB1 receptor deficiency on development of AD pathology were studied by knocking out CB1 receptor in AβPP23 mice of AD. AβPP23/CB1−/− mice showed worsen cognitive deficits than AβPP23 mice, thus suggesting that CB1 deficiency can worsen AD-related learning and memory deficits [111]. Moreover, an eCB re-uptake inhibitor, VDM-11, reversed Aβ-induced hippocampal damage and memory impairment in passive avoidance test [101]. Further research in this field revealed that cannabidiol treatment was able to prevent Aβ-induced memory impairments in rats and that CB1, but not CB2, receptors may be directly involved in improving Aβ-induced memory impairments and intrinsic electrophysiological properties of hippocampal pyramidal neurons [151]. Fakhfouri and colleagues [140] showed that administration of the synthetic cannabinoid agonist, Win55,212-2, significantly improved memory functions and decreased the elevated levels of neuroinflammatory markers like TNF-a, active caspase-3, and nuclear NvxB. Antagonist experiment confirmed that these neuroprotective effects of Win55,212-2 were partially mediated by CB1 and CB2 receptors [140]. Through CB1 receptor, Win55,212-2 increased PPAR-
pathway by increasing its transcription activity and provided neuroprotection [140]. Furthermore, the effects of cannabinoids were studied in transgenic murine models of AD. Prolonged oral treatment of CB2 receptor agonist (JWH-133) was able to improve cognitive impairments and decrease microglial activation in Tg2576 mice, while Win55,212-2 was ineffective [141]. Moreover, both cannabinoids significantly reduced the expression of CB2 receptor, TNF-α and COX-2 suggesting a critical role of CB2 in inflammatory processes in AD [141]. Recently, it has been shown that long-term treatment with cannabidiol was able to prevent the development of social recognition deficits in the AβPP/PS1 mouse model of AD [170]. The authors further revealed that these effects were not associated with decreased Aβ plaque load or oxidative changes while they noticed subtle effects induced by cannabidiol on neuroinflammation and cholesterol levels [170]. Moreover, a different study conducted on the same model showed that a combined treatment with cannabidiol and Δ⁹-THC reduced learning impairment, decreased soluble Aβ42 peptide levels and caused a change in plaques composition [171]. However, there are few reports that do not support beneficial effects of cannabinoids in AD treatment. Chen and colleagues found that chronic administration of the cannabinoid agonist HU-210 to AβPP/PS1 double transgenic mice did not improve water maze performance or a contextual fear conditioning task [172]. HU-210 neither altered AβPP processing and neuritic plaque formation nor enhanced hippocampal neurogenesis in AβPP/PS1 transgenic mice. It has been reported that CB1 blockade by rimonabant improved Aβ-induced memory impairments in mice tested in a passive avoidance paradigm. The authors suggested that such memory improvement might be due to the increased acetylcholine release in the brain [173].

Additional effects of cannabinoids

Apart from aforementioned mechanisms, few cannabinoids exert their therapeutic effects in similar way of currently US-FDA approved drugs for AD treatment. Most of the drugs currently used in AD treatment (donepezil, rivastigmine, and galantamine) are inhibitors of AChE. AChE is involved in degradation of neurotransmitter acetylcholine (ACh), which is reduced in AD [174]. Active component of marijuana, Δ⁹-THC, has been demonstrated to competitively inhibit AChE and to thus increase ACh levels [175]. Moreover, Δ⁹-THC prevented AChE-induced aggregation of Aβ which can reduce plaques formation [175]. In addition to Δ⁹-THC, other CB agonists also showed to have AChE and butyrylcholinesterase inhibition properties [176]. Alternative strategies based on multiple targets such as CB receptors and cholinesterase with single compound is gaining acceptance for treatment of AD. Besides AChE inhibitors, current AD treatment includes memantine, a NMDA receptor antagonist, which reduces excitotoxicity by inhibiting Ca²⁺ influx. In similar way, HU-211 (synthetic cannabinoid devoid of CB1 and CB2 agonist activity) protects neurons from excitotoxicity by antagonizing NMDA receptors [177–179]. Moreover, recently it was demonstrated that eCBs can modulate Aβ-induced alterations in Notch signaling. Notch signaling plays a pivotal role in neurodevelopment, and it is also involved in control of neurogenesis, neurite growth, synaptic plasticity, and long-term memory [180, 181]. In advance neurogenesis, Notch signaling is reduced [180]. Long term spatial deficits were observed in Notch mutant mice [182]. It has been shown that Aβ negatively regulates Notch-1 signaling by increasing expression of Numb, the endogenous negative regulator of Notch-1 cleavage [183]. Interestingly, AEA, through CB1 receptors, was able to reverse this effect by increasing expression of Numb, and Notch intracellular domain, Hes1 and Hes5 (see Fig. 1). Moreover, AEA and 2-AG were also able to inhibit Aβ-induced expression of Numb [183]. Furthermore, cannabinoids could provide beneficial effects by modulating cerebral blood flow functions. AD is characterized by a decreased regional cerebral blood flow that could result in decrease brain supply of oxygen, glucose, and nutrients. Cannabinoids can improve blood flow to the brain as CB1 receptor activation can elicit vasodilatation [184]. Moreover, as discussed earlier, cannabinoids can increase Aβ clearance at blood brain barrier [112]. CB2 receptor activation has been shown to improve blood-brain barrier integrity by decreasing adhesion of leukocytes to endothelial cells under inflammatory conditions [185], which may reduce further exaggeration of inflammation. However, besides beneficial effects, cannabinoids (especially at high doses) may exert unwanted cannabimimetic and psychiatric side effects such as hypolocomotion, hypothermia, aversion, and anxiety-related behaviors [186–189]. Moreover, CB1 receptor activation may precipitate episodes of psychosis and panic while its inhibition may lead to depression.
and anxiety-related disorders (for more details, see [190]). Furthermore, CB1 agonists may worsen AD by inhibiting acetylcholine release in the brain [7]. CB2 agonist and inhibitors of endocannabinoid deactivating enzymes seem to be devoid of such side effects. Therefore, much attention has been focused on this kind of compounds as potentially useful for the AD treatment.

CONCLUSIONS

The advances in AD research in the last decade have revealed that this disease is multifaceted in nature and is linked to different multiple mechanisms in the brain. A novel, more effective therapeutic approach for AD treatment should target multiple mechanism of disease progression. A large body of evidence suggested the involvement of the eCB system in the neurodegenerative process associated with AD. Aβ deposition in the brain is linked to significant changes in the expression pattern of CB2 receptors and FAAH enzyme. CB2 receptors and FAAH are selectively and abundantly overexpressed in microglia and astrocytes, respectively, in vicinity of Aβ neuritic plaques. AEA and its precursor N-arachidonylethanolamine (NarPE) levels are decreased in frontal cortex. In contrast, 2-AG degrading enzymes MAGL and ABHD6 activity is reduced in plaques and surrounding area. Over all AEA signaling is lowered and 2-AG signaling is increased in the vicinity of plaques. CB1 receptors expression in AD is still controversial and brain region specific. Although results of different groups are sometimes conflicting, a decline in the eCB system activity in AD is probable. This review proposes cannabinoids as potential therapeutics, which can target simultaneously neurodegeneration, neuroinflammation, oxidative damage, cognitive impairments, and clearance of Aβ from the brain. Figure 3 summarizes the beneficial effects...
of cannabinoids in AD treatment. Elevation of CB receptor activity either by pharmacological blockade of enzymes responsible for eCBs degradation or by direct receptor agonist could be a promising strategy for slowing down the progression of AD and alleviating its symptoms. Although increased CB2 expression and hydrolyzing FAAH activity is well documented in human AD patients as well as animal models of AD, a combination therapy of CB2 agonist and FAAH inhibitor did not receive much research attention. This combination therapy could potentially lead to more effective treatment for AD, as they would target the altered eCB signaling in AD patients and could thereby reduce neuro-inflammation through reduced pro-inflammatory eicosanoids production and microglial activation. However, treatment with FAAH inhibitors should be done with caution as FAAH knockout astrocytes showed exaggerated inflammation [137].

Endogenous or exogenous cannabinoids, through cannabinoid receptors and/or PPAR control the activity of various signaling pathways like MAPK, NfκB, Notch-1, and Wnt/β-catenin pathways. Through these pathways, cannabinoids could reduce inflammation, generation of Aβ plaques, and NFTs resulting in improvement of synaptic structure, synaptic plasticity, and learning and memory deficits. However, the pharmacological modulation of eCB signaling should be done considering the disease stage.

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