Endocannabinoid signalling in Alzheimer's disease

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Abstract

The ECs (endocannabinoids) AEA (anandamide) and 2-AG (2-arachidonoylglycerol) and their lipid congeners OEA (*N*-oleoylethanolamide) and PEA (*N*-palmitoylethanolamide) are multifunctional lipophilic signalling molecules. The ECs, OEA and PEA have multiple physiological roles including involvement in learning and memory, neuroinflammation, oxidative stress, neuroprotection and neurogenesis. They have also been implicated in the pathology of, or perhaps protective responses to, neurodegenerative diseases. This is particularly the case with Alzheimer's disease, the most common age-related dementia associated with impairments in learning and memory accompanied by neuroinflammation, oxidative stress and neurodegeneration. The present mini-review examines the evidence supporting the roles that ECs appear to play in Alzheimer's disease and the potential for beneficial therapeutic manipulation of the EC signalling system.

What are endocannabinoids?

ECs (endocannabinoids) are neuromodulators and immunomodulators derived from AA (arachidonic acid), phosphatidylethanolamine and diacylglycerol [1]. The principal ECs known to date are AEA (anandamide) and 2-AG (2-arachidonoylglycerol), with 2-AG being the most abundant form [2,3]. Both AEA and 2-AG are members of the larger classes of lipid signalling molecules NAEs (*N*-acylethanolamines) and MAGs (monoacylglycerols). In addition to the ECs, these classes encompass a number of ECLs (EC-like ligands), which include OEA (*N*-oleoylethanolamide) and PEA (*N*-palmitoylethanolamide) [4]. Since the latter are substrates for the same catabolic enzymes as the ECs, ECLs are thought to preserve and facilitate the activity of AEA and 2-AG, as well as possessing important biological functions of their own [5,6].

EC signalling

CB_1/CB_2 (cannabinoid 1 and 2) receptors signal mainly through the $G_{i/o}$ class of heterotrimeric G-proteins [7]. Activation of CB_1 or CB_2 receptors through binding of ECs or synthetic agonists, such as HU210, results in the dissociation of the G_α subunit from $G_{\beta\gamma}$ and the consequent initiation of a number of intracellular signalling cascades and modulation of membrane ion channels. ECs and ECLs are also able to bind and activate TRPV1 (transient receptor potential vanilloid type 1) ion channels [6,8] and nuclear PPARs (peroxisome-proliferator-activated receptors) [9].

Key words: Alzheimer's disease, endocannabinoid, learning and memory, neuroinflammation, neuroprotection.

Abbreviations used: AA, arachidonic acid; Aβ, amyloid β-peptide; ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; APP, amyloid precursor protein; BBB, blood–brain barrier; CB, cannabinoid; CNS, central nervous system; EC, endocannabinoid; ECL, EC-like ligand; ECS, EC system; FAAH, fatty acid amide hydrolase; 4-HNE, 4-hydroxy 2,3-nonenal; IL, interleukin; MAG, monoacylglycerol; MAGL, MAG lipase; nAChR, nicotinic ACh receptor; NFT, neurofibrillary tangle; NMDA, *N*-methyl-D-aspartate; OEA, *N*-oleoylethanolamide; PEA, *N*-palmitoylethanolamide; PPAR, peroxisome-proliferator-activated receptor; ROS, reactive oxygen species; Δ^9-THC, Δ^9-tetrahydrocannabinol.

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EC functions

Retrograde signalling

Perhaps the best-established function of ECs is their role as retrograde messengers acting through presynaptic CB_1 receptor activation to suppress neurotransmitter release [10]. As a consequence, ECs are intimately associated with the phenomena of LTP (long-term potentiation) and LTD (long-term depression), two alternative forms of synaptic plasticity underlying learning and memory [11].

Anti-inflammatory mediators

ECs and ECLs act as anti-inflammatory mediators by activating CB_2 receptors and PPARs [12,13]. Studies have shown that CB_2 receptor expression is up-regulated in activated microglia during neuroinflammation [14] and CB_2 receptor agonists have been shown to attenuate BBB (blood–brain barrier) dysfunction by reducing BBB permeability, partly through an increase in tight junction protein expression [14]. AEA has also been shown to prevent synthesis of biologically active pro-inflammatory cytokines IL (interleukin)-12 and IL-23 [15].

Antioxidants

Phenolic cannabinoids [Δ^9-THC (Δ^9-tetrahydrocannabinol), cannabinol, cannabidiol, CP 55,940, HU210 and AM 404] have also been shown to display antioxidant properties in a CB_1 receptor-independent manner [16]. Further support for a role for cannabinoids in oxidative stress is provided by a recent study indicating that, in addition to their roles as CB_1/CB_2 receptor agonists, HU210 and WIN55,212-2 are able to inhibit NADPH oxidase [17] and the associated generation of ROS (reactive oxygen species).

CB_2-receptor-selective stimulation has also been shown to reduce oxidative stress in neurons by inducing the expression of Bcl-2 and Hsp70 (heat-shock protein 70), while concomitantly promoting neuroprotection by preventing glial activation [18].

Neuroprotection

The neuroprotective effects of cannabinoids, ECs and ECLs have been well documented [19,20]. AEA has been shown to confer CB_1-receptor-dependent neuroprotection in a rat model of excitotoxicity [21], and CB_2 receptor activation by the synthetic CB_2-receptor-selective agonist JWH-015 is able to prevent remote cell death through prevention of cyctochrome *c* release from mitochondria [20]. Additionally, the neurons of CB_1 receptor$^{-/-}$ mutants are more susceptible to neurodegeneration induced by excitotoxic cell death than those from wild-type [22]. OEA [13] and PEA [19] have also been shown to provide neuroprotection following neuronal insult.

As a consequence of the multiple roles described above, ECs are beginning to be considered as viable treatment options for neurodegenerative disorders, including AD (Alzheimer's disease).

General background of AD

AD dementia is a progressive age-related neurodegenerative disorder affecting approximately 33.9 million people worldwide with the incidence estimated to triple in the next 40 years [23].

Neuropathology of AD

AD is characterized by the presence of extracellular Aβ (amyloid β-peptide) plaques and intracellular NFTs (neurofibrillary tangles) caused by hyperphosphorylation of the microtubule-associated protein tau [24]. Aβ is derived from proteolytic cleavage of APP (amyloid precursor protein) by β- and γ-secretase to produce Aβ_{40} and Aβ_{42} [24].

Inflammation in AD

A key feature of the AD brain is neuroinflammation caused by activated microglia releasing a host of compounds which activate astrocytes and cause effects such as excitotoxicty and the generation of ROS. Microglia are the phagocytic immune cells of the brain and they are activated early in the process of AD during which they take on anti-inflammatory roles [25]. However, activated microglia appear to be unable to remove or prevent the formation of Aβ plaques, and, indeed, microglial function appears to be inhibited by amyloid [26]. Additionally, T-cells infiltrating the CNS (central nervous system) promote the release of the anti-inflammatory cytokine IL-4 from astrocytes. IL-4 causes microglia to adopt an alternative activation phenotype in AD which promotes the release of anti-inflammatory agents and neurotrophic factors [25].

Excitotoxic insult

Neuroinflammation, in addition to direct effects of Aβ, causes the release of glutamate from neurons and glia. Glutamate activates NMDA (*N*-methyl-D-aspartate) receptors on neurons resulting in an influx of Ca^{2+}, thereby increasing the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) to pathological levels. Additionally, Aβ is capable of forming pores in the lipid bilayer which are permeable to Ca^{2+}, thereby further enhancing $[Ca^{2+}]_i$. Excessively raised $[Ca^{2+}]_i$ leads to cellular depolarization, mitochondrial dysfunction and generation of ROS.

Oxidative stress in AD

Oxidative stress is thought to occur early in the course of AD and is manifest in the formation of toxic compounds, such as peroxides [27]. Lipid peroxidation results in the generation of a number of toxic species, including the highly reactive 4-HNE (4-hydroxy-2,3-nonenal), levels of which are raised in AD [27]. 4-HNE has multiple detrimental effects, including impairing the uptake of glutamate by astrocytes, depleting membrane phospholipids, inhibition of neuronal glutamate and glucose transporters, Na^+/K^+-ATPases and dysregulation of Ca^{2+} homoeostasis [27].

Cholinergic transmission in AD

ACh (acetylcholine) is one of the primary neurotransmitters in the brain and has key roles in a number of processes, including memory and executive functions. ACh activates two classes of receptors: muscarinic and nicotinic. In AD, the expression of the presynaptic nAChRs (nicotinic ACh receptors) is reduced [28]. It has been suggested that Aβ is able to bind directly to nAChRs, in particular the α_7 subtype, with high affinity and to inactivate them [28].

ECs and AD

EC actions

ECs have been shown to have anti-inflammatory and anti-oxidant actions, as well as protecting against excitotoxicity and enhancing neurogenesis. The main mechanisms involved in AD are described above. As ECs are able to have an impact upon all of these systems to various extents, they may potentially have beneficial effects in AD [29].

Evidence supporting a role for the ECS (EC system) in AD

Support for the involvement of the ECS in AD pathology is provided by a number of human studies, which show up-regulation and nitrosylation of CB_1 and CB_2 receptors on microglia in the post-mortem AD brain [30]. CB_1 receptor expression was reduced, whereas CB_2 receptor expression was enhanced, in post-mortem AD brains [30,31], and cortical CB_2 receptor expression was correlated with Aβ_{42} levels and senile plaque score. In contrast, in a different study, no differences in CB_1 receptor levels were found in post-mortem AD brains [32], but a correlation was observed between frontal cortical CB_1 receptor levels and cognitive test scores assessed in the year prior to death. Additionally, it was demonstrated that CB_1 receptors in these patients'

brains were functionally intact and may have played a role in the preservation of cognitive function [32].

Levels of AEA and its lipid precursor NArPE (1-stearoyl,2-docosahexaenoyl-*sn*-glycerophosphoethanolamine-*N*-arachidonoyl) were also found to be reduced in the mid-frontal and temporal cortices of AD patients in post mortems [33]. Furthermore, AEA levels in these patients were found to be inversely correlated with $A\beta_{42}$, but not $A\beta_{40}$, and positively correlated with measures of speed of information processing and language abilities [33], although no differences in 2-AG levels were observed.

Raised levels of the acylethanolamides' catabolic enzyme FAAH (fatty acid amide hydrolase) are also found in brain areas containing plaque pathology [34]. In normal brains, FAAH immunoreactivity is primarily detected in neuronal elements, whereas, in AD brains, hypertrophied astrocytes show the most intense staining [34]. Astrocytic FAAH is hypothesized to degrade AEA, thereby releasing AA and resultant pro-inflammatory eicosanoids into the surrounding area, leading to an exacerbation of inflammation [1]. These changes are more pronounced in the vicinity of the plaques and are observed less frequently, or not at all, at more distant sites. FAAH inhibition has therefore been suggested as a logical therapeutic strategy for reducing AD-related neuroinflammation [34]. However, when testing this hypothesis by incubating astrocytes derived from wild-type and $FAAH^{-/-}$ animals with Aβ, surprisingly an increase in the expression of pro-inflammatory cytokines by $FAAH^{-/-}$ astrocytes was observed [35]. This effect, however, was not reproduced using pharmacological blockade of FAAH [35], thereby providing no corroborative support for the underlying proposal of beneficial effects of FAAH inhibition.

In addition to FAAH deletion, genetic deletion of MAGL (MAG lipase), the main enzyme responsible for 2-AG degradation, has also been proposed to have beneficial effects in AD [36]. This proposal was based on the observation that MAGL controls the generation from 2-AG of arachidonic acid, which is also the precursor of eicosanoids, including the prostaglandins. Genetic deletion of MAGL in an APP/PS1 (presenilin 1) double-transgenic mouse model of AD resulted in reduced eicosanoid levels, attenuated glial activation and associated neuroinflammation and reduced amyloid plaque burden [36].

Aβ-induced hippocampal degeneration and cognitive deficits are accompanied by increased synthesis of 2-AG, leading to the proposal that neuronal damage up-regulates EC synthesis [37]. This has prompted the suggestion that the production of ECs and the resultant CB receptor activation may be an attempt by the CNS to protect against damage [38]. Further support for this theory is provided by the observation that intraperitoneal administration of the membrane EC transport inhibitor VDM-11 to rodents previously injected with Aβ in the frontal cortex alleviated memory impairments and attenuated the resultant neurotoxicity [37]. However, the exact timing of VDM-11 administration was critical and, in order for it to have any beneficial effects, it had to be administered early in the disease process. Given later in the course of AD, it actually impaired memory retention, possibly as a result of reducing hippocampal ACh levels [38].

CB_1 $receptor^{-/-}$ mutants show enhanced central release of ACh, prompting the suggestion [39] that a degree of tonic inhibition of ACh release mediated by the ECs must normally exist in the CNS. In contrast, it has been shown that the intravenous administration of a small amount of CB receptor agonist, such as WIN-55,212-2, could also increase the release of ACh, which was reduced by the CB_1 receptor antagonist rimonabant [40]. In this instance, up-regulation of the ECS may be beneficial in AD, particularly in the light of the fact that one of the main treatments for AD involves the use of AChE (acetylcholinesterase) inhibitors. CB_2 receptor levels are raised in microglia in AD brains by neuroinflammation [29]. The synthetic cannabinoid receptor agonists HU210, WIN55,212-2 and JWH133 were able to return activated microglia to their resting state morphology *in vitro* in which they no longer secrete pro-inflammatory cytokines [30]. Additional effects attributed to CB_2 receptor include down-regulation of the CD40 ligand which may be involved in Aβ deposition and inhibition of NO (nitric oxide) production [1]. Oral administration of the CB_2-receptor-selective agonist JWH133 for 4 months rescued recognition memory impairments in 11-month-old AD mice in addition to normalizing cerebral glucose metabolism, as measured by FDG (2-[^{18}F]fluoro-2-deoxy-D-glucose)-PET (positron-emission tomography), and counteracting microglial activation [41].

Inhibition of NO generation by inhibiting iNOS (inducible NO synthase) reduces lipid peroxidation and is thought to be part of the mechanism by which cannabinoids function as antioxidants. Furthermore, it has been suggested that NO may be involved in the development of plaques and NFTs, and that its inhibition would influence multiple pathological parameters [42].

Another feature of AD that can be modified by cannabinoids is excitotoxicity. A number of studies have shown that activation of CB_1 receptors reduces excitotoxic cell death of hippocampal neurons by a number of mechanisms [43]. Under normal circumstances, one of the primary roles of the ECS is the regulation of neuronal ion channels [4]. Hence, cannabinoids acting through CB_1 receptors located in the vicinity of the NMDA channel are able to attenuate its activation and reduce Ca^{2+} influx. Since NAPE-PLD (*N*-acyl-phosphatidylethanolamine-specific phospholipase D; the major AEA synthetic enzyme) is Ca^{2+}-dependent, elevations in Ca^{2+} levels would be predicted to increase generation of ECs which feedback negatively to reduce transmitter release. In support of these concepts, CB_1 receptor agonists have previously been shown to reduce glutamate release by attenuating presynaptic Ca^{2+} entry [43]. The synthetic cannabinoid analogue HU211 is also able to prevent excitotoxicity, probably by direct inhibition of NMDA receptors. Furthermore, cannabinoids are able to act on microglial CB_2 receptors to inhibit their activation and, hence, prevent excess glutamate release, thereby reducing excitotoxic neuronal death in AD [30].

Cannabinoids are also involved in adult neurogenesis in the subventricular zone and dentate gyrus of the hippocampus. Neurogenesis is defective in animal models of AD, although post-mortem examination of human AD brains indicates an elevation in neurogenesis, perhaps as a response to on-going neurodegeneration [44]. Goncalves et al. [45] suggested that it is the activity of the CB_2 receptor in conjunction with that of 2-AG's synthetic enzyme diacylglycerol lipase which is of primary importance in neurogenesis. Hence, it may be possible to manipulate the ECS to enhance adult neurogenesis in a bid to replace the neurons lost in AD, and potentially attenuate the rate of cognitive decline.

Exogenous cannabinoids and AD

In addition to ECs, a number of exogenous cannabinoids including the constituents of *Cannabis sativa* have also been shown to have beneficial effects in AD. These include the phytocannabinoid cannabidiol [46], the major psychoactive component of *C. sativa* Δ^9-THC [47], and a pharmaceutical formulation of Δ^9-THC, dronabinol [48].

In vitro, cannabidiol has been shown to protect PC12, N13 and rat primary microglial cells from Aβ-induced neurotoxicity through a combination of anti-apoptotic, anti-oxidative and reduced intracellular Ca^{2+} influx [41]. These effects were shown to be mediated by the inhibition of the phosphorylated form of p38 MAPK (mitogen-activated protein kinase) and the transcription factor NF-κB (nuclear factor κB) [49]. *In vivo* studies examining the effects of cannabidiol treatment prior to intrahippocampal injections of Aβ into 3–5-month-old mice supported *in vitro* studies by demonstrating attenuated measures of inflammation and oxidative stress [50]. The same group subsequently showed the neuroprotective effects of cannabidiol to be mediated by PPARγ activation [46], although other groups have suggested a role for the cannabinoid and adenosine A_{2A} receptors [41].

In addition to cannabidiol, studies have also demonstrated potential beneficial effects of Δ^9-THC in AD. As stated above, one of the main mechanisms for treating AD currently is through the use of AChE inhibitors. Δ^9-THC has been shown to competitively inhibit AChE through its peripheral anionic site whereby it serves a dual function in preventing ACh metabolism and also diminishing Aβ aggregation [47].

In addition to its role in AChE inhibition, dronabinol has been shown to be effective in stimulating appetite and alleviating disturbed behaviour in patients with a clinical diagnosis of probable AD who were refusing food [48].

Summary

In summary, ECs may act as a double-edged sword in the pathophysiology of AD. Activation of CB_1 receptors has been shown to exacerbate memory impairments unless it occurs within a narrow spatial and temporal window [37]. In contrast, the support of neurogenesis and the anti-inflammatory actions associated with CB_2 receptor activation may be of benefit. Hence, blockade of CB_1 receptors concomitantly with enhanced CB_2 receptor activation could provide a feasible treatment option for attenuation and possibly reversal of AD pathology. Additionally, as cannabis smoking is linked to memory impairments, it is possible that CB_1 receptor antagonism may provide some short-term benefits, even in the absence of the ECS having a pathological role in AD.

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