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The Endocannabinoid System and Alzheimer's Disease

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Abstract The importance of the role of the endocannabinoid system (ECS) in neurodegenerative diseases has grown during the past few years. Mostly because of the high density and wide distribution of cannabinoid receptors of the CB_1 type in the central nervous system (CNS), much research focused on the function(s) that these receptors might play in pathophysiological conditions. Our current understanding, however, points to much diverse roles for this system. In particular, other elements of the ECS, such as the fatty acid amide hydrolase (FAAH) or the CB_2 cannabinoid receptor are now considered as promising pharmacological targets for some diseases and new cannabinoids have been incorporated as therapeutic tools. Although still preliminary, recent reports suggest that the modulation of the ECS may constitute a novel approach for the treatment of Alzheimer's disease (AD). Data obtained *in vitro*, as well as in animal models for this disease and in human samples seem to corroborate the notion that the activation of the ECS, through the use of agonists or by enhancing the endogenous cannabinoid tone, may induce beneficial effects on the evolution of this disease.

Keywords Endocannabinoid system · Alzheimer's disease · Animal models

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Introduction

The ECS is currently seen as a neuromodulatory system participating in several important biological functions. In the brain, the massive presence and wide distribution of the type 1 cannabinoid receptor (CB_1) explain many of the effects (reviewed in previous articles of this monograph) of the cannabinoid chemicals as well as those of the "endocannabinoids", the endogenous ligands that, naturally present in the body, bind and activate these receptors. The CB_1 receptor has been, for the last 15 years, the main target for the development of new cannabinoid-related therapeutic agents. However, the psychotropic effects derived from its activation have been a serious handicap for their actual translation into the clinic.

In vitro and *in vivo* studies have shown that exogenous and endogenous cannabinoids are neuroprotective. Although the precise mechanism(s) supporting this effect has not been completely unveiled, it is now known that involves CB_1 (i.e., decreases in glutamate release, production of pro-inflammatory molecules and $[Ca^{2+}]_i$, and stimulation of GABAergic transmission), CB_2 (i.e., anti-inflammation) and non receptor-mediated (i.e., antioxidative) mechanisms (for a recent review, see [1]). Some of these recent studies are summarized in Tables 1 and 2 and are indicative of a putative therapeutic action of these compounds in conditions of acute and chronic neurodegeneration.

Among different models of chronic neurodegeneration, the usefulness of cannabinoids has been postulated for Huntington's disease (HD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and, more recently, Alzheimer's disease (AD). AD is one of the most prevailing neurological diseases in Western countries, with more than four million people affected only in the United States and accounting for the

Table 1 Cannabinoids are neuroprotective *in vitro*

Reference	Insult	Ligand(s)	Receptor Mediation	Sample
Shen and Thayer (Mol Pharmacol 54, 459–462, 1998)	Mg2+ induced excitotoxicity	WIN 55,212-2	CB_1-mediated	Hippocampal neurons (rats)
Nagayama et al. (J Neurosci 19, 2987–2995, 1999)	Hypoxia and glucose deprivation	WIN 55,212-2	Non-CB_1-mediated	Cortical neurons (rats)
Hampson et al. (PNAS 95, 268–273, 1999)	Direct excitotoxicity	CBD, THC	Non-CB_1-mediated	Cortical neurons (rats)
Sinor et al. (Neurosci Lett 278, 157–160, 2000)	Hypoxia and glucose deprivation	AEA, 2-AG	Non-CB_1-mediated	Cortical neurons (rats)
Abood et al. (Neurosci Lett 309, 197–201, 2001)	Direct excitotoxicity	THC	CB_1-mediated	Mice (spinal cord)
Skaper et al. (PNAS 93, 3984–3989, 1996)	Direct excitotoxicity	PEA	CB_1- / CB_2-mediated	Mice
Marsicano et al. (J Neurochem 80, 448–456, 2002)	Oxidative stress	THC, WIN	Non-CB_1-mediated	Cell lines Mice
Chen et al. (Mol Brain Res 134, 215–225, 2005)	NMDA	THC	Non CB_1-mediated	AF5 cell line
Lastres-Becker et al. (Neurobiol Dis 19, 96–107, 2005)	6-OH-DA	HU-210	Not studied	Cerebellar granule cells (mice)
Karanian et al. (J Neurosci 25, 7813–7820, 2005)	AMPA	Methanandamide AM374 AM404	CB_1-mediated	Rat brain slices
Fernández-López et al.(Ped Res 60, 169–173, 2006)	Oxygen-glucose deprivation	WIN	CB_1- / CB_2-mediated	Newborn hippocampal slices (rats)
Melis et al. (Neurobiol Dis 24, 15–27, 2006)	Ischemia	2-AG	CB_1-mediated	Rat brain slices
Gilbert et al. (Brain Res 1128, 61–69, 2007)	Low Mg^{2+} levels	WIN THC	CB_1-mediated	Rat hippocampal neurons

most frequent form of dementia in the elderly. Furthermore, it is expected that more than 14 million people may have the disease by year 2050, if no preventive treatment (s) become available [1, 2].

Although still controversial, the most widely accepted theory on the pathogenesis of AD suggests that the deposition of amyloid-β protein (Aβ) in specific areas of the brain is the seminal event of a long-lasting series of changes that lead to a massive neuronal and neurotransmitter loss, with accompanying symptoms (such as memory deficits, cognitive and motor impairment, etc). Three main pathological hallmarks have been clearly defined for AD [3] and include loss of functional synapses and appearance of intracellular neurofibrillary tangles (mostly composed by hyperphosphorylated tau protein) and extracellular neuritic plaques (enriched in Aβ). Aβ deposition triggers local neuroinflammatory responses in which astroglia and, remarkably, microglia, play crucial roles [3–5].

Unfortunately, current treatments for AD only provide symptomatic relief. The cholinesterase inhibitors are being widely used although with limited success, thus indicating the need for new therapeutic approaches. Donepezil, rivastigmine, and galantamine are among this type of drugs, capable of increasing acetylcholine levels in the synaptic cleft and are indicated for the mild and moderate stages of the disease [6]. More recently, the *N*-methyl-D-aspartate glutamatergic receptor antagonist memantine has been incorporated to the clinical practice, being prescribed for the treatment of moderate to severe AD. The possible usefulness of its combined administration with cholinesterase inhibitors is currently being investigated [6]. In addition, anti-Aβ therapies are currently thought as some of the most promising strategies for the treatment of AD, and even clinical trials with vaccines against this peptide have been carried out, although dampened by the appearance of some cases of encephalitis [7]. The possible interest of antiinflammatory compounds is also under debate [8], and clinical trials have been performed. In particular, nonsteroidal antiinflammatory drugs, cyclooxigenase-2 (COX-2) inhibitors and peroxisome-proliferator-activating-receptor ligands have been tested (reviewed in [8]).

It is also important to note that many groups have reported on the critical role that brain inflammation plays in AD ([4, 9, 10]). Our current understanding indicates that neuroinflammation is an early event in the pathogenesis of AD and that may be triggered by Aβ deposition. In turn, an inflammatory milieu together with microglial activation

Table 2 Cannabinoids are neuroprotective *in vivo*

Reference	Insult	Ligand(s)	Receptor Mediation	Sample
Nagayama et al. (J Neurosci 19, 2987–2995, 1999)	Global ischemia	WIN 55,212-2	CB_1-mediated	Rats
Van der Stelt et al. (J Neurosci 21, 6475–6479, 2001; J. Neurosci 21, 8765–8771, 2001)	Direct excitotoxicity	THC, AEA	CB_1-mediated Non CB_1-mediated (early vs late)	Rats
Panikashvili et al. (Nature 413, 527–531, 2001; J Cereb Blood Flow Metab 25, 477–484, 2005)	Closed head injury	2-AG	CB_1-mediated	Mice
Hansen et al. (J Neurochem 82, 1–5, 2002)	Direct excitotoxicity	SR141716A	CB_1-mediated	Newborn rats
Parmentier-Batteur (J Neurosci 22, 9771–9775, 2002)	Focal ischemia Direct excitotoxicity	Not studied	CB_1-mediated	KO mice
Martínez Orgado et al. (Mol Brain Res 114, 132–139, 2003)	Ischemia plus hypoxia	WIN 55,212-2	CB_1-mediated Non CB_1-mediated (early vs late)	Newborn rats
Marsicano et al. (Science 302, 84–88, 2003)	Direct excitotoxicity	UCM707	CB_1-mediated	KO Mice
Panikashvili et al. (J Cereb Blood Flow Metab 25, 477–484, 2005)	Closed head injury	2-AG	CB_1-mediated	KO mice
Lastres-Becker et al. (Neurobiol Dis 19, 96–107, 2005)	6-OH-DA	THC CBD	Non-CB_1-mediated	Rats
Karanian et al. (J Neurosci 25, 7813–7820, 2005)	AMPA	Methanandamide AM374 AM404	CB_1-mediated	Rats
Melis et al. (Neurobiol Dis 24, 15–27, 2006)	Ischemia	2-AG	CB_1-mediated	Rats
Shouman et al. (British J Pharmacol 148, 442–451, 2006)	Ibotenic acid s-bromowillardiine	AEA	CB_1-mediated	Mice Rats
Panikashvili et al. (Neurobiol Dis 22, 257–264, 2006)	Trauma	2-AG	Not studied	Mice
Garcia-Arencibia et al. (Brain Res 1134, 162–170, 2007)	6-OH-DA	CBD AM404 HU308	CB_2-mediated	Rats

seem to enhance tau hyperphosphorilation, thus aggravating the damaging process. Data obtained in animal models [11, 12] as well as in human AD patients [13] have recently confirmed this observation.

ECS and AD: *In Vitro* Studies

As mentioned above, both exogenous and endogenous cannabinoids exert neuroprotective functions in different models of neuronal damage. *In vitro* studies have largely confirmed this notion. Thus, it was of interest to explore the effects of ECS modulation after exposure to Aβ (see Table 3). Milton [14] reported that anandamide (AEA) and noladin ether are potent inhibitors of Aβ-toxicity *in vitro*. By using a human nervous cell line (Ntera 2/cl-D1), these data were the first to show a counteracting effect of these endocannabinoids on Aβ toxicity. Interestingly, both ligands were effective at nanomolar concentrations and their effects were exerted through a CB_1-dependent, MAPK-mediated mechanism, as AM-251 and PD98059, an antagonist for this type of cannabinoid receptor and an inhibitor of the MAPK pathway, respectively, completely prevented their neuroprotective action.

Recent *in vitro* studies have added important features to the role of the ECS in AD. In particular, a prominent role for microglial CB_2 receptors is emerging [15, 16]. These reports clearly show that CB_2 activation leads to an inhibition of Aβ-induced microglial activation. Experiments performed by Ramirez et al. [16] in primary cultures demonstrated that: 1) the CB_1 agonist, HU-210, was capable of preventing Aβ1-40-induced changes in microglial morphology; 2) CB_1 (HU-210 and WIN55,212-2) and CB_2 (WIN55, 212-2 and JWH-133) agonists significantly inhibit the microglial production of TNF-α, a cytokine known to participate in Aβ-triggered damage; and 3) the use of conditioned media allowed these authors to conclude that those cannabinoids exerted their neuroprotective action *in vitro* solely through microglia and not directly on neurons.

The prominent role of CB_2 receptors on *in vitro* microglial function after exposure to Aβ was further confirmed by Ehrhart et al. [15]. These authors explored the effect of CB_2 activation on the expression of the CD40 receptor, known to participate in the microglial response to Aβ. Interestingly, they found that the CB_2 agonist JWH-015 induced a decrease in CD40 expression by mouse microglial cells in primary culture after exposure to interferon-gamma and prevented the Aβ-triggered production of proinflammatory cytokines. Further, microglial phagocytosis was potentiated by this agonist by attenuating CD40-mediated inhibition. Current experiments from our laboratory seem to confirm the activational role of CB_2

Table 3 Cannabinoids and Aβ (*in vitro* data)

Reference	Insult	Ligand(s)	Receptor Mediation	Effect	Involved Pathways	Sample
Milton (Neurosci Lett 332, 127–130, 2002)	Aβ (several, mostly Aβ1-40)	AEA Noladin-ether	CB_1-mediated	↓ MTT	MAPK	NT-2 neurons
Ramírez et al. (J Neurosci 25, 1904–1913, 2005)	Aβ25-35 and Aβ1-40	HU-210 WIN55,212-2 JWH-133	CB_1-mediated CB_2-mediated	↓ Morphological changes ↓ MTT ↓ TNF-α ↑ Neuronal survival	–	Mouse primary culture (microglia and neurons)
Ehrhart et al. (J Neuroinflm 2, 29–42, 2005)	Aβ1-42	JWH-015	CB_2-mediated	↓ IFN-γ-mediated CD40 expression ↓ TNF-α ↓ NO ↑ Phagocytosis	JAK/STATI	Mouse primary culture (microglia)
Iuvone et al. (J Neurochem 89, 134–141, 2004)	Aβ1-42	Cannabidiol	No	↓ MTT ↓ ROS ↓ Lipid peroxidation ↓ Caspase 3 ↓ DNA fragmentation ↓ $[Ca^{2+}]_i$	–	PC12
Esposito et al. (J Mol Med 84, 253–258, 2006)	Aβ1-42	Cannabidiol	No	↓ Tau hyper-P ↓ GSK-3-P	Wnt/β-catenin	PC12
Esposito et al. (Neurosci Lett 399, 91–95, 2006)	Aβ1-42	Cannabidiol	No	↓ iNOS ↓ NO	p38MAPK NF-κB	PC12
Esposito et al. (Neurosci Lett 404, 342–346, 2006)	Aβ1-42	WIN55,212-2 ACEA	CB_1-mediated	↓ iNOS ↓ NO	–	C6

ligands on cell phagocytic activity against Aβ (Núñez et al., in preparation). These data suggest that microglial CB_2 receptors may be postulated as novel targets for the modulation of the response against Aβ deposition [16]. It is important to note that this receptor type has been described in primary microglial cells as well as in different microglial cell lines and its expression is up-regulated after pro-inflammatory stimuli [17].

In addition to the above described CB_1- and CB_2-mediated effects on *in vitro* cell responses against Aβ, other nonreceptor-mediated roles of cannabinoids have been shown. Iuvone's group in Naples [18, 19, 21–23] has provided exciting results on the actions of cannabidiol, a nonpsychotropic natural cannabinoid. From their contributions, it can be concluded that cannabidiol: 1) exerts a neuroprotective role in rat pheocromocytoma PC12 cells, and 2) this action is caused by a combination of antioxidant and anti-apoptotic mechanisms, as well as to the modulation of the p38MAPK, NF-κB and Wnt/β-catenin pathways (see Table 3).

ECS and AD: Studies in Animal Models

Few studies have explored so far the role of the ECS in AD *in vivo*, and all of them have used Aβ administration directly into the CNS as experimental paradigm. Memory loss is one of the earliest and more frequent symptom of AD and, on the other hand, the memory-disrupting effects of cannabinoids are well characterized (see [24] for review). Thus, it was reasonable to think that CB_1 blockade might improve memory after Aβ exposure. These notions led Mazzola et al. [25] to explore the effect of the blockade of CB_1 receptors on the amnesia induced by Aβ1-42 and Aβ25-35 (i.c.v.) in mice. Using the passive avoidance test, these authors observed that the CB_1 antagonist SR141716A was capable of preventing the amnesia induced by both types of amyloid peptides. Based on previous studies on the effects of cannabinoid agonists on acetylcholine release, they speculated that SR141716A might produce this benefitial effect by enhancing the cholinergic hippocampal transmission. In contrast with this hypothesis, a very recent report from Eubanks et al. [26] has shown that delta-9-tetrahydrocannabinol (THC) is able to inhibit acetylcholinesterase and to prevent the Aβ aggregation induced by that enzyme by directly interacting with the peripheral anionic site of the protein.

Contradictory to these behavioral data, Ramírez et al. [16] reported that the cannabinoid agonist WIN55,212-2 prevented the cognitive impairment induced by Aβ25-35 i.c.v. administration to Wistar rats. This effect might be explained by the neuroprotection induced by this agonist, as revealed by the quantification of the neuronal markers calbindin and tubulin. In addition, microglial activation was also prevented by WIN55,212-2 administration. It is currently well-

known that microglia play a pivotal role in the response to amyloid deposition, modulating the immunological response of the brain. These microglia also secrete a wide variety of pro-inflammatory molecules that ultimately trigger neuronal damage in AD. Thus, based on these data, it seems reasonable to think that the reported prevention of microglial activation also collaborates in the neuroprotective effect of WIN55,212-2 in this rat model of AD.

Van der Stelt and colleagues [20] have recently reported exciting data on the modulation of the ECS in an animal model of AD. By administering the soluble form of the pathogenic amyloid peptide (Aβ1-42) in the cortex of rats and mice, these authors studied: 1) the effect of Aβ1-42 administration on hippocampal levels of AEA and 2-arachidonoyl-glycerol (2-AG); and 2) the effect of the enhancement of the ECS on the evolution of the pathological disease and on memory processing. Their results show that, as in other models of brain insult [27, 28], endocannabinoid levels were significantly elevated as a consequence of toxic damage to the brain, although only 2-AG levels were sensitive to Aβ1-42 administration. Interestingly, no change in CB_1 receptor expression was detected 12 days after damage induction, whereas a significant increase in CB_2 expression was found at that time point. Although paradoxical when considering the massive loss of hippocampal neurons (shown by decreased calbindin levels), the absence of changes in CB_1 expression is in concordance with data reported in other pathological conditions. In parallel, selective CB_2 up-regulation seems to corroborate the inducible nature of this receptor type, as previously suggested [17, 29, 30].

Furthermore, these authors also provided data on the effect of the enhancement of the endocannabinoid tone on Aβ1-42-induced brain damage and memory impairment, by using the uptake inhibitor VDM-11. Their results suggest that early, but not late, potentiation of the endocannabinoid tone leads to neuronal loss prevention (as measured by recovery of calbindin levels) and to an antiinflammatory response (determined by decreases in COX-2 and iNOS expression). Notably, memory impairment was reduced after VDM-11 administration on day 3 after peptide injection into the cortex, whereas memory loss was aggravated when the same compound was administered on day 7. Consequently, these authors conclude that a robust and early potentiation of the endocannabinoid tone may be beneficial for the prevention of Aβ1-42-induced neurodegeneration *in vivo*.

It is important to note that cannabinoids have been also reported to be beneficial in other animal models of neuroinflammation. Very recently, Marchalant et al. [31] have shown that WIN55,212-2 decreases the microglial activation induced by chronic infusion of bacterial lipopolysaccharide (LPS). These authors, however, did not find any improvements in locomotor activity. In addition, cannabinoid receptors of the CB_1 type were not detected in microglial or astroglial cells, the presence of these receptors being restricted to neurons and thus pointing to an indirect action of the cannabinoid agonist on LPS-treated rats.

More animal studies are needed to calibrate the contribution of the ECS to the development and treatment of AD *in vivo*. In particular, the use of transgenic models of AD and knockout animals for the different elements of the ECS may be powerful tools to achieve this objective.

ECS and AD in Humans

As mentioned in the "Introduction," their psychoactive properties have deeply limited human studies with cannabinoid chemicals and related compounds. In respect to AD patients, their possible use as appetite stimulants and antiemetics has attracted considerable attention, although with contradictory results [32–35]. One study showed a positive effect of dronabinol on body weight increase accompanied by improvements in disturbed behaviors, partially because of decreased agitation [35]. Very recently, Walther et al. [36] performed a pilot study on five AD patients and reported that a low dose of dronabinol was able to significantly improve several clinical parameters (such as nocturnal motor activity, agitation, etc), without undesired side effects.

However, recent data have opened new perspectives in the area [37]. The analysis of human postmortem brain samples from AD patients has provided information on the neuropathology of the ECS that rises new hypothesis on the possible role of this system in the prevention and/or treatment of AD. These data may be summarized as follows: 1) Aβ deposition induces dramatic changes in the phenotype of glial cells, including up-regulation of some elements of the ECS (such as CB_2 receptors and FAAH) [29]; 2) CB_1 receptor binding in hippocampus and basal ganglia structures is decreased in AD brains [38], but not in neocortex or frontal cortex [16, 38] and they are less efficiently coupled to signal transduction mechanisms [16]; 3) overexpression of CB_2 and FAAH seems to be a phenomenon directly linked to Aβ deposition, as the study of human samples of a natural model of AD suggests, like Down's syndrome (Núñez et al, submitted); 4) the up-regulation of these elements of the ECS is a cell-specific event, as CB_2 seems to be restricted to microglia and FAAH to astrocytes, and 5) the activation and prevalence of a glial ECS under neuroinflammatory conditions in the human CNS has been also observed for other diseases, such as HIV-induced encephalitis (Benito et al, unpublished; [39]) and multiple sclerosis [40].

Of special relevance may be the induction of the expression of CB_2 receptors in microglial cells. It has only been recently accepted that this type of cannabinoid receptor may be present in the CNS, as previous work circumscribed its

presence to peripheral cells and tissues of the immune system [41]. Although the functions of these receptors in the CNS are far from clear, they may now be considered as diagnostic markers for microglial activation and as relevant candidates for the development of anti-Aβ therapies. The amount of *in vitro* data in the literature on the antiinflammatory effects of CB_2 activation, for instance, rises appealing possibilities, as antiinflammatory compounds are under intense study as putative useful agents for the treatment of AD patients.

The modulation of FAAH expression and activity also constitutes an interesting approach. Our current hypothesis suggests that FAAH inhibition may provide benefits for dampening the local inflammatory process triggered by Aβ as it would render more endocannabinoids available for interaction with their receptors. Together with the observed decrease in CB_1 binding and functional coupling in human AD samples [16, 38], the putative psychoactive effects derived from the potentiation of the endogenous cannabinoid tone could have a lower impact. In addition, a decrease in FAAH expression and/or activity would also affect the local levels of arachidonic acid, one of the products of FAAH enzymatic activity on AEA, and precursor of a series of potent pro-inflammatory mediators.

Conclusions

Neurologists agree that, in its early phase, AD is not easy to diagnose. Subtle memory deficits and cognitive impairments may have a slow evolution until the disease becomes evident. Unfortunately, once it is diagnosed, only symptomatic treatments are available and these show limited efficacy. Thus, the search for new preventive and, more importantly, curative therapies is increasingly demanding. Agents capable of modulating the activity of the ECS may constitute novel and promising therapeutics, mainly because: 1) exogenous and endogenous cannabinoids protect neurons from excitotoxicity and other types of insults, and 2) the activation of CB_2 receptors and the blockade of endocannabinoid-degrading enzymes may potentiate the neuroprotection and trigger antiinflammatory responses. The use of CB_2 and FAAH-selective agents opens new exciting possibilities and avoids the pitfalls derived from the psychoactive properties of cannabinoids.

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