The Role of the Endocannabinoid System in Alzheimer's Disease: Facts and Hypotheses

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Abstract: Unlike other neuroinflammatory disorders, like Parkinson’s disease, Huntington’s disease and multiple sclerosis, little is still known of the role of the endocannabinoid system in Alzheimer’s disease (AD). This is partly due to the poor availability of animal models that are really relevant to the human disease, and to the complexity of AD as compared to other neurological states. Nevertheless, the available data indicate that endocannabinoids are likely to play in this disorder a role similar to that suggested in other neurodegenerative diseases, that is, to represent an endogenous adaptive response aimed at counteracting both the neurochemical and inflammatory consequences of β-amyloid-induced tau protein hyperactivity, possibly the most important underlying cause of AD. Furthermore, plant and synthetic cannabinoids, and particularly the non-psychotropic cannabidiol, might also exert other, non-cannabinoid receptor-mediated protective effects, including, but not limited to, anti-oxidant actions. There is evidence, from in vivo studies on β-amyloid-induced neurotoxicity, also for a possible causative role of endocannabinoids in the impairment in memory retention, which is typical of AD. This might open the way to the use of cannabinoid receptor antagonists as therapeutic drugs for the treatment of cognitive deficits in the more advanced phases of this disorder. The scant, but nevertheless important literature on the regulation and role of the endocannabinoid system in AD, and on the potential treatment of this disorder with cannabinoids and endocannabinoid-based drugs, are discussed in this mini-review.

INTRODUCTION

Auguste D., who died in 1906, was the first reported person to whom doctor Alzheimer diagnosed the clinical condition now well known as Alzheimer's disease (AD). As the most common age-related neurodegenerative disorder, AD currently has a prevalence of 10% in individuals over 65 years and its significant impact on the worldwide health care system will continue to increase with the increasing longevity of the population. AD is a chronic neurodegenerative condition clinically characterized by a progressive loss of cognitive abilities and, eventually, dementia. It can be classified into two forms: 1) sporadic AD, which accounts for the majority of cases, and 2) the rarer, familial early-onset form (FAD), in which mutations of genes encoding, for example, amyloid beta precursor protein (APP) and presenilin-1 and -2 [1,2], have been suggested to underlie the development of the disease. The presence of senile neuritic plaques and neurofibrillary tangles (NFTs), the increased oxidative damage to lipids, proteins and nucleic acids and the loss of biometal homeostasis are the main features of the AD brain. The senile plaques are extracellular insoluble aggregates of β-amyloid (Aβ), a cleavage product of the larger transmembrane protein, APP. NFTs are intracellular lesions consisting of paired helical filaments due to hyperphosphorylation of the microtubule-associated protein tau that otherwise, when physiologically phosphorylated, maintains integrity of the cytoskeleton by promoting the assembly and stability of microtubules. As mentioned, the homeostasis of some transition metals such as copper, iron and zinc, is also altered in the AD brain, although it is not clear if this phenomenon is a cause or a consequence of AD. In particular, high levels of biometals have been found in Aβ aggregates, and metal dys-homeostasis may contribute to the unbalance in the activity of tau kinases and phosphatases responsible of NFT formation.

Since the etiology of AD is still not fully clear, many hypotheses, including increased expression of apoptotic proteins [3,4], impaired ubiquitin-proteasome system [5,6], oxidative stress (OS) [7,8], inflammation [9], altered energy metabolism [10] as well as cholinergic, dopaminergic and serotonergic dysfunctions [11,12], have been investigated. Although five drugs are currently approved by the FDA for the treatment of AD symptoms, there is still no effective treatment available for this disorder. In particular, cholinesterase inhibitors (i.e. Donepezil, Galantamine, Rivastigmine and Tacrine) and the NMDA receptor antagonist, Memantine, are used in mild to moderate and in moderate to severe AD, respectively, even though Tacrine is now rarely used because of its hepatotoxicity [13]. In addition, antibodies against Aβ peptides [14], non-steroidal anti-inflammatory drugs (NSAIDs) [15] and PPARγ agonists [16] seem to be some of the most hopeful strategies for the development of new drugs to cure AD. In this scenario, increasing interest has been focused on the endocannabinoid system (EC), which is more and more being considered as a novel and promising source of pharmaceuticals for the treatment of several neurodegenerative disorders. The endocannabinoids, including anandamide, 2-arachidonoylglycerol, noladin ether, N-arachidonoyldopamine and virodhamine (Fig. 1), or...
Fig. (1). Chemical structures of endocannabinoids. Of the compounds shown here, only anandamide and 2-arachidonoylglycerol (2-AG) have been sufficiently studied to conclusively suggest their role as endogenous agonists of cannabinoid receptors.

synthetic agents that activate either or both of the best characterized endocannabinoid receptors, the cannabinoid CB1 and CB2 receptors, or plant cannabinoids, such as the CB1 and CB2 agonists Δ9-tetrahydrocannabinol (THC) and the weakly cannabinoid receptor-active cannabidiol, exert beneficial effects in several animal models of Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), Huntington’s disease (HD) and multiple sclerosis (MS) [17]. Such effects, ranging from neuro-protective, anti-neuroinflammatory and anti-oxidant actions (Fig. 2), have been studied, both in vitro and in vivo, also in models of Aβ-induced neurotoxicity, gliosis and microglial cell activation, which, however, are not necessarily relevant to the human AD. Nevertheless, such actions, which are exerted via both CB1/CB2-mediated and non-CB1/CB2-mediated mechanisms, will be reviewed in this article, together with so far much scatter evidence obtained in mouse transgenic models of AD. Furthermore, the capability of pathological components of AD to influence the expression of cannabinoid receptors and endocannabinoid metabolic enzymes, and to modulate the levels of endocannabinoids, both in vitro and in vivo, and in both rodents and humans, will be described, as this represents further evidence that the endocannabinoid system is part of the several adaptive responses aimed at attempting to re-establish homeostasis after neuroinflammatory and neurodegenerative insults.

**IN VITRO STUDIES ON CANNABINOID RECEPTOR-MEDIATED INHIBITION OF Aβ-INDUCED NEUROTOXICITY, GLIOSIS AND NEUROINFLAMMATION**

One of the first in vitro evidence of the involvement of the endocannabinoid system in this neurodegenerative disorder was reported by Milton [18]. Two endocannabinoids, anandamide and noladin ether, were tested against Aβ-induced neurotoxicity in a differentiated human teratocarcinoma cell line, the Ntera 2/c1-D1 neurons. Both compounds were able to inhibit Aβ toxicity via a CB1-mediated mechanism and activation of the mitogen activated protein kinase (MAPK) pathway, since both AM-251 and PD98059, a CB1 receptor antagonist and a MAPK inhibitor, respectively, reverted their neuroprotective action [18].

In another study carried out in primary cultures of murine microglial cells, the effect of selective stimulation of CB2 receptors on the inflammatory response induced by Aβ was investigated. The selective CB2 agonist, JWH-015, decreased both the expression of the cell surface antigen molecule, CD40, and the phosphorylation of Janus protein tyrosine kinase and signal transducer and activator of transcription-1, induced by interferon-γ (IFN-γ). Importantly, JWH-015 reduced the secretion of the pro-inflammatory molecules, tumor necrosis factor-α (TNF-α) and nitric oxide (NO), induced either by IFN-γ or Aβ, and was also able to attenuate the CD40-mediated inhibition of microglial phagocytosis of the Aβ1-42 peptide [19]. Also the Aβ-induced microglial activation in vitro, the induction of cell morphological changes and the enhancement of TNF-α production were reported to be reverted by pre-treatment with the dual CB1/CB2 agonists, HU-210 and WIN55,212-2, or with the selective CB2 agonist, JWH-133 [20]. The authors also suggested that the neuroprotective effect of cannabinoids observed in vivo (see below) was likely due to the prevention of Aβ-induced microglial activation and not to a direct action on neurons.

In another study, WIN 55,212-2 and the selective CB1 receptor agonist, arachidonoylchlorehanolamide (ACEA) were able to inhibit, via activation of CB1 receptors, the production of NO and the expression of inducible NO synthase (iNOS) caused by 24h stimulation of C6 rat glioma cells with Aβ (1-42), and to reduce tau protein hyperphosphorylation in differentiated PC12 neurons co-cultured with C6 cells [21]. Another set of experiments demonstrated that exposure of C6 cells to Aβ induced the modulation of the endocan-
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The above findings suggested that Aβ-induced neurotoxicity might be accompanied by elevated endocannabinoid levels in brain areas that, like the hippocampus, participate in memory retention. For this reason, the modulation of endocannabinoid levels after stereotactic injection of Aβ(1-42) was more recently investigated in rats by van der Stelt et al. [26], who indeed showed that this treatment was followed by a transient elevation of 2-AG and CB2 receptor levels in the hippocampus of treated rats 12 days after the injection, and by a reduction of anandamide levels 20 days after the injection [26]. No change was observed at any time in the cortex, which was the site of the stereotactic injection. The enhancement of 2-AG levels was accompanied by up-regulation of the 2-AG-biosynthesizing enzyme, diacylglycerol lipase-α (DAGLα). Furthermore, the pharmacological enhancement of endocannabinoid levels through the sub-chronic administration of an inhibitor of endocannabinoid-cellular reuptake, VDM-11, three days after Aβ injection, caused a dramatic reduction of those markers of neuronal apoptosis and gliosis, that had been caused by the Aβ peptide in the hippocampus. On the other hand, when VDM-11 administration was started only seven days after Aβ injection,
no effect was observed on these markers, even though the compound was still very efficacious at elevating hippocampal endocannabinoid levels. The authors also carried out similar experiments with VDM-11 in mice treated with Aβ (1-42) in a way similar to that reported in the study by Mazzola et al. [25]. Again, the compound reverted the memory retention deficits only when administered starting 3 days from Aβ peptide injection, and it worsened memory retention when administered 7 days after, whilst being in both cases equi-efficacious at elevating brain endocannabinoid levels [26]. The authors suggested that elevation of endocannabinoid levels is an early event after Aβ peptide-induced neurotoxicity, aimed at counteracting neuronal damage and gliosis, thus explaining why early pharmacological elevation of endocannabinoid levels with VDM-11 causes neuroprotection in rats and improves memory retention in mice. However, at later stages, elevation of endocannabinoid tone at CB1 receptors, possibly by interfering with acetylcholine release in the hippocampus, might instead contribute to the major sign of Aβ peptide-induced neurotoxicity, i.e. memory retention loss. This would explain why late administration of rimonabant [25] and VDM-11 [26] improve or worsen memory retention, respectively, and why no changes in 2-AG levels and even a decrease of anandamide levels are observed in the hippocampus 20 days following the injection of Aβ (1-42) in rats [26]. In at least partial agreement with this hypothesis was the earlier finding that the cannabinoid receptor agonist WIN55,212-2 prevented the cognitive deficits in the spatial navigation task occurring in Aβ-peptide-treated rats, and correspondingly exerted neuroprotective actions by preventing microglial activation and neuronal death [20]. Based on in vitro data (see above), these effects were suggested to be mediated by CB2 receptors, which all authors agree as being elevated following treatment with Aβ peptides. On the other hand, Esposito et al. [22] later showed that activation of CB2 receptors, as opposed to CB1 receptor stimulation, can be deleterious for Aβ (1-42)-induced neurotoxicity in vivo in rats. These seemingly discrepant results might be explained if one bears in mind that: 1) the inflammatory response due to microglial activation in neurodegenerative disorders plays first a protective action, while it contributes to neuronal damage only when it becomes chronic and/or if it is accompanied by the disruption of the blood brain barrier; and 2) activation of CB2 receptors has been suggested to play both anti- and pro-inflammatory effects, depending on CB2-induced chemotaxis and inhibition of cytokine release, respectively. Also the stimulatory effect of CB2 on immune cell motility, depending on its exact localization, might either attract or take away macroglia, astrocytes, neutrophils, mast cells, lymphocytes and macrophages from the site of inflammation [27]. Therefore, it is possible that initial neuroprotective effects of endocannabinoids are exerted by activating CB1 receptors, for example via reduced excitotoxicity. Instead, in this initial phase, activation of CB2 receptors might be deleterious as it might block the early repair process mediated by microglial cells. By converse, in a later phase of the disorder, activation of CB1 receptors might contribute to memory retention loss, whereas stimulation of CB2 receptors might tone down the excessive macroglial activation and the subsequent inflammatory response caused by macrophage and lymphocyte infiltration from the circulation [28].

**STUDIES IN TRANSGENIC MICE**

A transgenic model of the familiar form of disease was used to study the possible influence of environmental factors (e.g. stress, exercise, enrichment) in the development of AD later in life. Double transgenic TASTPM mice, which overexpress the APP and the presenilin-1 genes, were subjected to a mild stress procedure consisting in the repeated exposure to an unfamiliar clean cage (novel cage), 4 times a week, over a period of weeks [29]. Since repeated stress is known to down-modulate endocannabinoid and CB1 levels in brain limbic areas [30], it could be hypothesized that such procedure might indirectly affect the performance of mice in memory tests. Therefore, a contextual fear conditioning test was used to assess short-term memory performance [31], and the brain regional levels of soluble and insoluble amyloid as well as of endocannabinoids were quantified [29]. Novel cage exposure attenuated soluble and insoluble amyloid accumulation in the hippocampus and frontal cortex, and it did so without affecting the age-related increases in regional brain endocannabinoid levels. These beneficial effects were interpreted by the authors as being likely due to the increase in physical and exploratory activity induced by novel cage exposure, and not to changes in endocannabinoid tone.

**STUDIES ON NON-CANNABINOIDS RECEPTOR-MEDIATED INHIBITION OF Aβ-INDUCED NEUROTOXICITY, GLIOSIS AND NEUROINFLAMMATION**

Evidence on a promising protective effect of cannabidiol, the principal non-psychoactive component of *Cannabis sativa*, against Aβ-induced neurotoxicity has been recently published. Cannabidiol does not bind to CB1 or CB2 receptors in vitro at concentrations lower than 5 μM, and is nevertheless a potent anti-oxidant [32] and anti-inflammatory agent. It exerts these effects mostly via non cannabinoid receptor-mediated mechanisms that might involve several other types of receptors as well as direct interactions with enzymes, including those that regulate endocannabinoid levels [33]. In differentiated rat pheocromocytoma cells (PC12) exposed to fragment 1-42 of the Aβ peptide, cannabidiol reduces the production of neuroinflammatory mediators by modulating the activity of p38 MAPK, nuclear factor-kB (NF-kB) and iNOS [34,35]. It also prevents tau hyperphosphorylation through the Wnt/β-catenin pathway [36]. Importantly, the anti-inflammatory effect of cannabidiol was confirmed also in vivo in the mouse hippocampus, where the compound was able to negatively modulate GFAP transcription and expression and to reduce interleukin-1β and iNOS up-regulation [37].

Two possible non-cannabinoid receptor-mediated mechanisms that involve p53 activation as responsible of the protective action of the two synthetic ligands, CP55,940 and JWH-015, on Aβ− (25-35) and H2O2-induced apoptosis in peripheral blood lymphocytes, have been recently reported [38]. The authors proposed that the two compounds might act via: 1) an anti-oxidant effect, with subsequent inhibition of Aβ-generated H2O2; and 2) activation of NF-kB and down-regulation of p53 via phosphoinositide 3-kinase (PI3K).

Another non-cannabinoid receptor-mediated effect was reported for THC, which was recently found to competi-
tively inhibit acetylcholine esterase (AchE)-induced aggregation of Aβ by interacting with the peripheral anionic binding site of the enzyme, the main region thought to be responsible for amyloidogenesis [39]. This finding might prompt new molecular modelling studies aimed at exploiting the chemical structure of THC for the design of more potent inhibitors of AchE-induced Aβ aggregation.

**HUMAN AND CLINICAL STUDIES**

Only few human studies have been reported so far on the involvement of the endocannabinoid system in AD. The immunohistochemical analysis of postmortem brains from patients with AD revealed an upregulation of both CB2 receptors and the endocannabinoid-degrading enzyme, fatty acid amide hydrolase (FAAH) in glial cells associated with senile plaques [40]. The presence of the degradative enzyme was limited to astrocytes, whereas CB1 was expressed only in activated microglial cells. On the other hand, CB1 receptor density was found to be unchanged in the proximity of neuritic plaques [40]. In an earlier study, no correlation had been found between changes in CB1 receptor protein or mRNA levels in the AD brain and the specific histopathological features of the disease [41]. In yet another study, a reduction of CB1-receptor-positive neurons in areas of microglial activation of the AD brain, along with enhanced nitration of both CB1 and CB2 receptors, were observed by using again immunohistochemistry [20]. Moreover, [35S]-GTPγS binding stimulated by WIN55,212-2, a measure of G-protein coupling and, therefore, of CB1 receptor functional activity, was greatly diminished in samples from AD patients [20]. The subsequently impaired signalling might be peculiar of CB1 since it had been reported previously that, unlike Gs-, Gi- protein mediated signaling remains preserved in AD [42], and might suggest that CB1 receptor agonists may not be as efficacious as expected in AD patients.

Down’s syndrome (DS) is sometimes referred to as a human model of Alzheimer-like Aβ aggregation, since, by the age of 40, virtually all patients with this syndrome have sufficient Aβ plaques for AD diagnosis [43,44]. Recently, a relationship between the appearance of Aβ and the expression of FAAH and CB2 was again suggested based on data form DS brains obtained from donors at different ages [45]. In particular, the authors reported that the presence of Aβ-enriched plaques increased with age in DS samples together with the up-regulation of both FAAH and CB2 expression in astroglia and microglia, respectively. CB2 expression, mostly restricted in neurons, remained, instead, unchanged.

Despite the above information on the state of the endocannabinoid system in the human AD brain, no studies have been carried out to ascertain the clinical potential of endocannabinoid-based drugs in this disorder, perhaps also because of the limited pharmacological and biochemical data available in transgenic models of AD. The assessment of the possible protective effects of cannabinoid receptor agonists on AD progress and symptoms might be soon become possible due to the fact that the administration of Dronabinol (a pharmaceutical preparation based on THC) for six weeks has been recently reported as very useful for the treatment of both the severity of disturbed behaviour and anorexia in food-refusing patients with AD and other dementias [46]. This study might open the way to easier to interpret, double-blind, placebo-controlled clinical trials, which might in turn give the opportunity to determine if such treatments have only a palliative importance or might also impact on the disease progress. On the other hand, perhaps also based on the observation from animal studies that endocannabinoids acting at CB1 receptors might contribute to memory loss caused by Aβ peptides [25,26], AVE1625, a selective CB1 receptor antagonist, is being tested in a double-blind, placebo-controlled phase II clinical trial in patients with mild to moderate AD (Clinical Trials.gov Identifier: NCT00380302). The compound is being tested at daily doses of 10 mg and 40 mg for 12 weeks, to assess its safety and tolerability and its effects on cognition, global functioning and behavior at week 12, following physical examination and neurological assessment with vital sign monitoring and electrocardiograms. The study has been completed but the results have not been disclosed yet.

**CONCLUSIONS**

As evidenced in this article, still little is known regarding the regulation and role of the endocannabinoid system in AD, and on the possible targeting of this system as a therapeutic strategy to treat this disorder. The limited data available indicate that the endocannabinoids are likely to play in this disorder a role similar to that suggested for other neurodegenerative diseases (Fig. 2), i.e. to represent an endogenous adaptive response aimed at counteracting, via both CB1 and CB2 receptors, both the neurochemical and inflammatory final consequences of amyloid β-induced tau protein hyperactivity - in fact, no evidence exists to date to suggest that activation of cannabinoid receptors might counteract specifically those aspects of AD that are peculiar of this disorder. However, apart from similarities, also differences between the role played by endocannabinoids in AD and other neurodegenerative disorders might exist (see [47,48] for reviews). These data might open the way to the use, against AD progress, of CB1 and CB2 receptor direct agonists. Furthermore, “indirect” agonists, i.e. synthetic compounds that prolong the activation state of such receptors by inhibiting endocannabinoid degradation via FAAH or other catabolic enzymes, or via the putative endocannabinoid membrane transporter [49], might be as efficacious as, and safer than, direct agonists, as they might act via several receptor types and only in those brain areas where the endocannabinoids are being produced and degraded [26]. On the other hand, since the endocannabinoids appear to also contribute to the cognitive symptoms of Aβ-induced neurotoxicity, mostly via CB1 receptors, antagonists of these receptors might also be useful in the late phases of the disorder to reduce the cognitive deficits of AD. Finally, evidence exists to suggest that non-cannabinoid receptor-mediated mechanisms induced by anti-inflammatory components of Cannabis, first of all cannabidiol, might also be exploited in the future as relatively safe therapeutic strategies. Further studies in transgenic models of AD and in humans are clearly needed to fully appreciate the role and regulation of the endocannabinoid system, and its potential exploitation for the treatment of AD.

**ABBREVIATIONS**

Aβ = β-Amyloid
ACEA = Arachidonoylchloroethanolamide
AchE = Acetylcholine esterase
AD = Alzheimer's disease
2-AG = 2-Arachidonoylglycerol
ALS = Amyotrophic lateral sclerosis
AM-251 = 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-(1-piperidyl)pyrazole-3-carboxamide
APP = Amyloid precursor protein
AVE1625 = N-[bis(4-Chlorophenyl)methyl]-3-azetidinyl]-N-(3,5-difluorophenyl)-methanesulfonamide
CB1 = Cannabinoid receptor type 1
CB2 = Cannabinoid receptor type 2
CP55,940 = 2-[(1S,2R,5S)-5-Hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol
DAGLα = Diacylglycerol lipase alpha
DS = Down’s syndrome
EC = Endocannabinoid system
FAAH = Fatty acid amide hydrolase
FAD = Familial Alzheimer's disease
FDA = Food and Drug Administration
GFAP = Glial fibrillary acidic protein
HD = Huntington’s disease
HU-210 = 6a,10aR)-9-(Hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,10a-tetrahydrobenzo[c]chromen-1-ol
IFN-γ = Interferon gamma
iNOS = Inducible NO synthase
JWH-015 = (2-Methyl-1-propyl-1H-indol-3-yl)-1-napthalenylmethanone
MAPK = Mitogen activated protein kinase
MS = Multiple sclerosis
NF-kB = Nuclear factor-kB
NFT = Neurofibrillary tangle
NMDA = N-methyl-D-aspartic acid
NO = Nitric oxide
NSAIDs = Non-steroidal anti-inflammatory drugs
OS = Oxidative stress
PD = Parkinson’s disease
PD98059 = 2′-Amino-3′-methoxyflavone
PI-3K = Phosphoinositide 3-kinase

PPARγ = Peroxisome proliferator-activated receptor gamma
SR141716A = 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide
THC = (−)-Δ9-Tetrahydrocannabinol
TNF-α = Tumor necrosis factor alpha;
VDM-11 = N-arachidonoyl-(2-methyl-4-hydroxyphenyl) amine
WIN55,212-2 = (R)-(+)-2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl-1-naphthalenylmethanone

REFERENCES

References 50-52 are related articles recently published.
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