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**Review Article**

# The role of cannabinoids in preventing the neurodegenerative process occurring in Alzheimer's disease

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**CONTENTS**



**Abstract**

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder and accounts for at least 50% of dementia cases. During the last decades, great strides have been made in our understanding of the molecular and cellular events leading to the pathology of AD, and results of some preclinical studies have been promising. However, only a few therapies have been introduced into the clinic. The characterization of the cannabinoid system within the last years has led to the development of cannabinoid-based therapies for the treatment of various diseases. In particular, the neuroprotective effects of this class of drugs against acute brain damage and their antiinflammatory properties prompted us to study cannabinoid receptors in AD brains and their possible neuroprotective effects in both *in vivo* and *in vitro* models. We found that the functioning of cannabinoid receptors is dramatically reduced in AD brain tissue and that cannabinoids prevent β-amyloid peptide-induced neurotoxicity and cognitive decline in rats, effects which may be due to their ability to inhibit microglial activation both *in vivo* and *in vitro*. These findings may be the basis for the use of cannabinoids as a therapeutic approach for AD.

## Alzheimer's disease and its therapy

Treatment of neurodegenerative diseases represents a major challenge for medicinal chemists, neuropharmacologists and clinical neurologists. In particular, Alzheimer's disease (AD) currently affects around 15 million people worldwide. The risk of suffering AD varies with age: thus, the incidence increases from 0.5% per year at the age of 65 to 8% per year after the age of 85. Given that life expectancy has markedly increased in industrialized countries over the last century, today AD treatment and care constitutes a major social and health problem.

In AD, brain regions involved in learning and memory processes (*e.g.,* the hippocampal region and several cortical areas) are reduced in size, they present the pathological alterations characteristic of the disease and show loss of particular subsets of neurons. In view of the high incidence of AD, research efforts during the past few decades have focused on understanding the cellular and molecular events associated with the pathology of the disease. Animal models, human postmortem material and genetic analyses have all provided important clues to the etiology of AD and, in fact, the present search for effective therapies is based on these findings (1-3). There are two types of therapies: palliative, in which drugs aimed at symptom relief are used, and disease-modifying agents, that prevent and/or delay the onset or slow the course of the disease.

The first neurochemical deficit that was reported in AD was the dramatic reduction in the neurotransmitter acetylcholine (ACh), known to be involved in learning and memory. However, replacement therapy, which has been used successfully in other neurodegenerative disorders such as Parkinson's disease, has not been proven to be very effective. Therefore research focused on developing and testing inhibitors of acetylcholinesterase (AChE), the major ACh-degrading enzyme. Several of these inhibitors (tacrine, donepezil, rivastigmine) have been approved and introduced into clinical practice. Of the seven drugs currently approved by the FDA for the treatment of AD, all

but one have this pharmacological action (4). However, their efficacy is limited and in some instances their central and peripheral side effects are considerable. Galantamine, however, is showing promise because in addition to inhibiting AChE it also activates some subtypes of nicotinic receptors which control the release of other major neurotransmitters, and also enhances ACh release.

Although the majority of AD cases are sporadic and of unknown etiology, some are a consequence of genetic mutations. A small number of patients have mutations in the gene of the amyloid precursor protein (APP) or presenilin-1 and -2 (PS1 and PS2). These mutations result in increased levels of total β-amyloid (Aβ) or of the more pathogenic $A\beta_{1-42}$ (5). Aβ is generated by sequential proteolytic processing of APP. Aβ deposits in AD brains forming the senile plaques, one of the pathological markers of the disease, are a feature of both sporadic and familial AD. Aβ is toxic both *in vitro* and *in vivo.* Different neural cell types and cell lines in culture die upon exposure to Aβ through a mechanism that involves oxidative stress, disruption of calcium homeostasis and/or excitotoxicity. Furthermore, transgenic mice expressing human APP mutations progressively develop plaques and cognitive impairment in different behavioral tasks. Taken together, these observations led to the development of the amyloid cascade hypothesis of AD, that states that imbalance between Aβ production and clearance results in a cascade of events eventually leading to neuronal dysfunction and dementia. Therefore, many of the therapeutic strategies for AD are aimed at decreasing Aβ accumulation by preventing its synthesis or by increasing its removal (6). An example of the former is the family of inhibitors of β- and γ-secretases, the enzymes responsible for the abnormal cleavage of APP that results in amyloidogenic peptides (normal cleavage relies on α-secretase activity). Some drugs that had retrospectively been demonstrated to diminish the risk of suffering AD such as nicotine, nonsteroidal antiinflammatory drugs (NSAIDs) (7, 8) and statins (cholesterol-lowering drugs) (9, 10) have recently been shown to behave in this manner. However, the $A\beta_{42}$-lowering property is not a general characteristic of NSAIDs, and even some COX-2 selective inhibitors may raise $A\beta_{42}$ by targeting the gamma-secretase complex (11). Active and passive vaccination has been employed to target removal of Aβ, and in animals carrying the human mutations of APP (APP transgenic mice, APP Tg), it has been quite successful (12, 13) but clinical trials had to be interrupted because some cases of cerebral hemorrhage occurred. Interestingly, other compounds may increase the flux of Aβ from brain to the periphery, for example insulin growth factor I (14) and gelsolin (15), and also lower amyloid burden.

Inflammation is another feature almost always observed in AD brains. Microglial cells, the immune cells of the brain, play a major part in the process (16). There is overwhelming evidence that a vast number of inflammatory mediators are increased in AD (17). As mentioned above, NSAIDs decrease the risk of developing AD. Although several studies have shown that NSAIDs have no beneficial effects in AD patients, they may help to delay the onset of the disease. In a recent study, the statins were also reported to exhibit antiinflammatory properties (18) by inhibiting Aβ-induced expression of IL-1β and inducible nitric oxide synthase, as well as NO production by microglia and monocytes. Interestingly, these actions were independent of their cholesterol-lowering properties (18).

Other strategies have been aimed at decreasing neurodegeneration and enhancing neuroprotection (4, 6) by the use of antioxidants, glutamate antagonists and trophic factors. Oxidative stress, either as a primary cause or as a consequence of the ongoing neurodegenerative process, is involved in AD etiology as well (19). As mentioned above, Aβ-induced cell death is accompanied by oxidative damage in vitro and in transgenic mice in vivo (20). However, the evidence obtained in postmortem brain tissue from patients are thus far scarce and, in some cases, contradictory. These results are the rationale for studying the effects of different antioxidants in AD models, in which they prevent some of the pathological features of the disease. It should be noted that in diagnosed AD patients vitamin E was ineffective, again suggesting that prevention is the mechanism of their beneficial effect. Nerve growth factor (NGF), which is essential for maintaining cholinergic forebrain neurons, has been shown to be decreased in AD, and therefore mice with reduced NGF may serve as a model of the disease (21). A phase I clinical trial has recently reported the effectiveness of NGF delivery by fibroblasts implanted into the forebrain. After 22 months of follow-up, patients with mild AD showed improved rates of cognitive decline and PET scans with significant increases in cortical 18-fluorodeoxyglucose after treatment (22).

In summary, efforts have been made to establish useful therapies to treat AD, including two approaches: palliative treatment to slow its progress and preventive treatment to delay its onset. Preventive therapy should be introduced soon in the evolution of AD and even in asymptomatic individuals at risk of developing the disease (*e.g.,* subjects carrying mutations), since palliative treatment has been shown to be ineffective when neuron loss is severe as in advanced stages of AD.

## Cannabinoids as neuroprotective agents

Cannabinoids are the active components of the plant *Cannabis sativa*. $\Delta^9$-Tetrahydrocannabinol (THC) is the major cannabinoid of the plant due to its high abundance and potency, and at least 60 other cannabinoids have been characterized to date. Several synthetic analogues more potent than THC have been developed and various cannabinoid agonists are now available, including compounds chemically related to THC such as the tricyclic dibenzopyranes (*e.g*., HU-210) and others that have no structural similarity to THC such as the aminoalkylindoles (*e.g.,* WIN-55212-2) (Fig. 1). Finally, endogenous

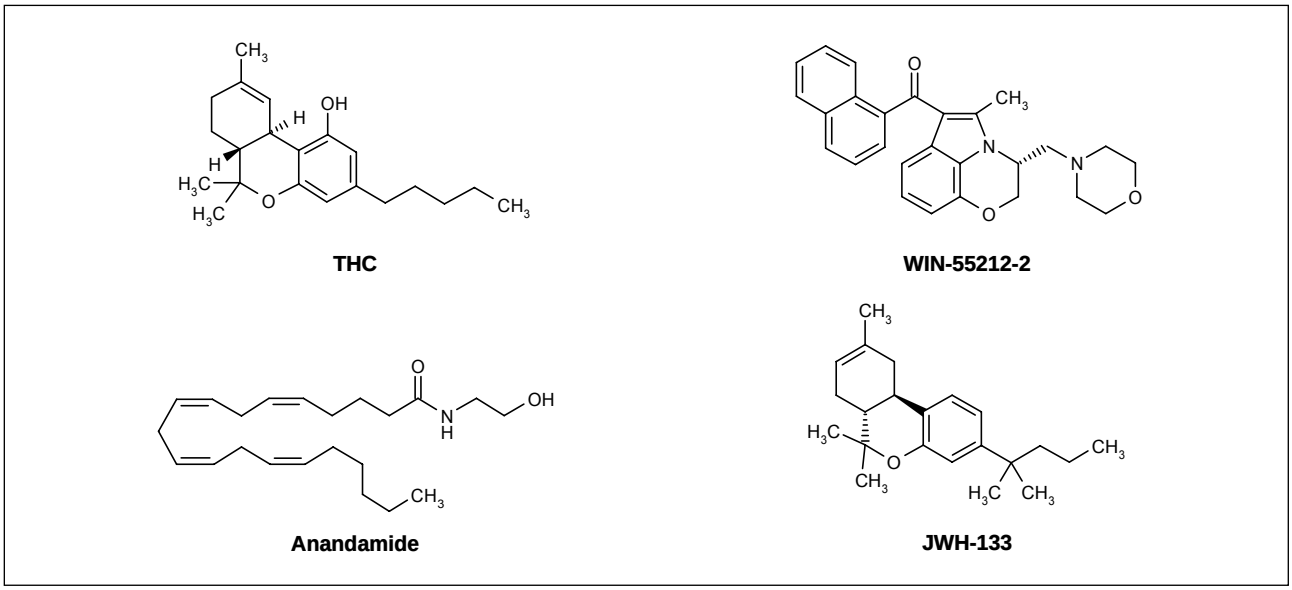


Fig. 1. Chemical structures of the cannabinoid agonists $\Delta^9$-tetrahydrocannabinol (THC), WIN-55212-2, anandamide and JWH-133.

cannabinoid molecules —the so-called endocannabinoids— have been discovered. These eicosanoid-like compounds are chemically related to arachidonic acid, two of which are anandamide (Fig. 1) and 2-arachidonoylglycerol. These molecules bind to CB cannabinoid receptors, two of which have been well characterized to date: $CB_1$ and $CB_2$ (23, 24). However, based on pharmacological evidence and on studies in knockout mice, the existence of more subtypes has been suggested (24). Fortunately, in addition to mixed $CB_1$/$CB_2$ agonists, selective agonists and antagonists are becoming available (25-27). The chemical structure of JWH-133, a $CB_2$ selective agonist that has 200-fold higher potency at $CB_2$ receptors compared to $CB_1$ receptors (28), is shown in Figure 1.

$CB_1$ receptors show a very high density and are widely distributed in the brain (23, 24, 29). Several brain regions are particularly enriched in $CB_1$ receptors, which are responsible for the neurofunctional effects of cannabinoids. Control of motor activity results from receptor activation in basal ganglia and cerebellum, learning and memory are affected through receptors expressed in cortical and hippocampal regions, amygdala cannabinoid receptor activation modulates emotions, and sensory control, including pain, is regulated by receptors located in the thalamus, spinal cord and peripheral nerve endings. The $CB_2$ receptor was considered to be uniquely expressed by cells and organs of the immune system until recently, and therefore was called the peripheral cannabinoid receptor, in contrast to the central cannabinoid receptor ($CB_1$) that is expressed mainly in the brain. However, $CB_2$ and $CB_2$-like receptors are also present in peripheral nerve endings, but are absent in normal brain. The presence of $CB_2$ receptors in microglial cells (*i.e.*, macrophage precursor cells residing in the brain) was long suspected and confirmed in culture assays (30). Cannabinoids modulate migration, cytokine expression and function in cultured microglia (30-33). $CB_1$ receptor activation appears to be responsible for some of the effects (33), while the $CB_2$ receptor mediates others (30).

Evidence for the neuroprotective role of cannabinoids has accrued over the past years. Indeed, cannabinoids are able to counteract the neurotoxicity induced by different types of insults in cultured neurons (34). Excitotoxicity induced by increased glutamate release from hippocampal, cortical or cerebellar cells is counteracted by cannabinoids (35-37). Cannabinoids modulate classical neurotransmitter release through $CB_1$ presynaptic receptors located at axon terminals and may lower glutamate extracellular levels reaching excitotoxic concentrations. The mixed cannabinoid agonist WIN-55212-2 provided neuroprotection of cortical neurons against hypoxia and glucose deprivation *in vitro* (38). In other paradigms, neuroprotection by cannabinoids was adscribed to their antioxidant effects due to their lack of affinity for CB receptors and the inability of selective antagonists to block the effect (35). Indeed, Marsicano *et al*. reported that cannabinoids exhibiting antioxidant properties in cell-free systems also protected $CB_1$-expressing cells, either transfected cells or primary neurons, against oxidative stress, while compounds devoid of antioxidant effects were inactive as neuroprotectants (39). Interestingly, other types of neural cells are also protected from cell death by cannabinoid receptor activation, such as the case of astrocyte death induced by ceramide (40) or sensitized to oxidative stress by trophic factor withdrawal (41), and oligodendrocytes undergoing apoptosis due to trophic factor removal (42). It should be noted that increased survival of those glial cells brought about by cannabinoids may have a positive impact on neuronal protection as well. In contrast, in some studies, cannabinoids have been shown to induce neuronal cell death. Different experimental factors may account for this opposite effect of cannabinoids, for example: a) dual

*Table I: Drugs targeted for treating Alzheimer's disease*.

| Alteration | Target | Drug type | References |
|---|---|---|---|
| ↓ACh | AChE | AChE inhibitors | |
| | Nicotinic receptors | Nicotine | |
| ↑Aβ synthesis | Synthetic enzymes | Secretase inhibitors, NSAIDs | 64 |
| | | Statins | 9, 10 |
| | Nicotinic receptors | Nicotine | |
| ↑Aβ deposition | Enhanced clearance | IGF-I, statins, vaccination | 10, 12-14 |
| | | Curcumin | 65 |
| | | Estrogens | 66 |
| ↑Inflammation | | NSAIDs, statins | 7-10, 18 |
| ↑Oxidative stress | Enzymes | Antioxidants, estrogens | |
| ↓Cell survival | TyrK receptors | Trophic factors | 22 |
| ↑Glutamate | Glutamate receptors | NMDA blockers | |

ACh: acetylcholine; IGF-I: insulin-like growth factor I; NMDA: N-methyl-D-aspartate; NSAIDs: nonesteroidal antiinflammatory drugs; TyrK: Tyrosine kinase.

effects may rely on the concentration and duration of exposure, with higher signal inputs usually increasing toxicity; b) type of experimental paradigm, in which the nature of the insult, the origin of the neuron or its stage of differentiation may define its vulnerability; c) several endocannabinoids also interact with other receptors that may balance the cell fate outcome. This is the case of AEA and arvanil, compounds that activate $CB_1$ receptors as well as type 1 vanilloid receptor (TRPV1), a member of the transient receptor potential channel family.

The neuroprotective effects of cannabinoids have also been investigated in different *in vivo* models and are summarized in Table I. The first study addressing this issue was that of Nagayama and coworkers (38). Cannabinoid receptor activation reduced hippocampal neuron loss following transient global cerebral ischemia and reduced infarct volume after permanent focal cerebral ischemia induced by middle cerebral artery (MCA) occlusion in rats (38). The protective effect was blocked by the selective $CB_1$ antagonist SR-141716 and the cannabinoid not only

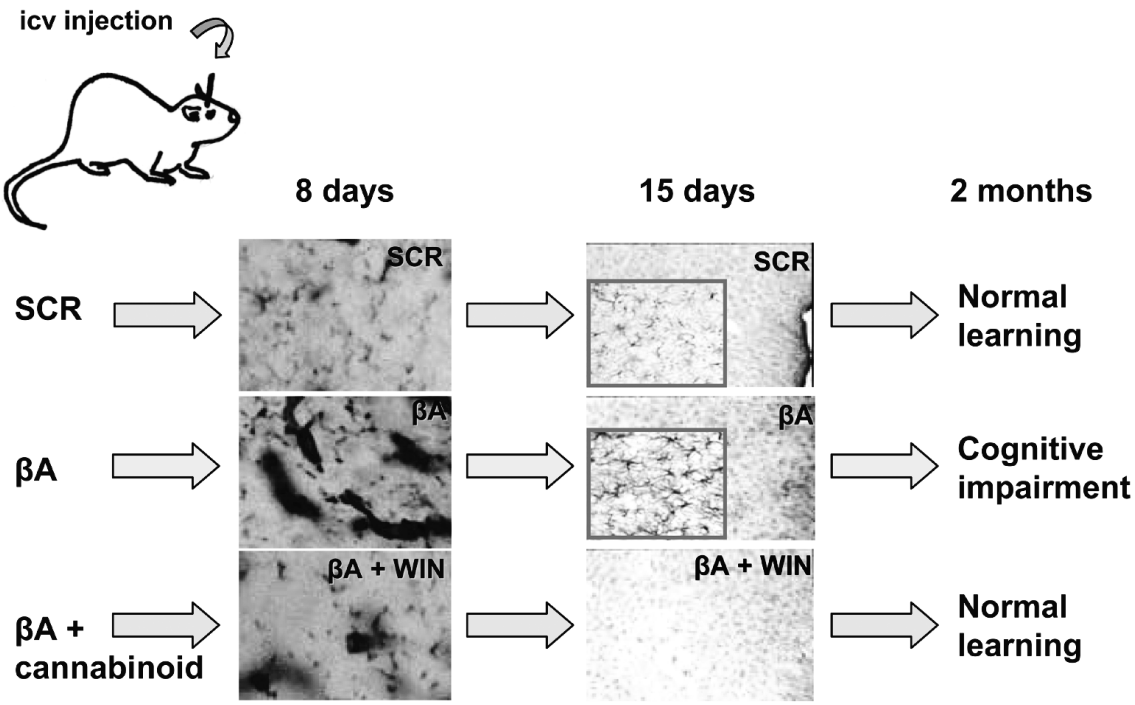


Fig. 2. Cannabinoids counteract the effects of Aβ administration *in vivo*. Rats were injected i.c.v. with Aβ (20 μg/rat/day for 7 days) alone or in combination with WIN-55212-2 (WIN; 10 μg/rat/day for 7 days). They were tested and sacrificed at the times indicated. Control rats received a control peptide ("scrambled" peptide, SCR). Aβ induced marked microglial activation at 8 days after beginning treatments and astrogliosis at 15 days, both of which were prevented by WIN administration. Aβ-induced impairment of spatial navigation at 2 months was prevented by cannabinoid injection as well.

afforded protection when given 30 min before MCA occlusion but also when administered 30 min afterwards, though not at later times (38). Furthermore, WIN-55212-2 given 10 min after submitting neonatal rats to asphyxia reduced early neuron loss and fully prevented delayed neuron loss, the latter in a $CB_1$-dependent manner (43). Neuroprotection by cannabinoids was monitored against ouabain-induced excitotoxicity in rats. THC, AEA and arvanil all afforded neuroprotection as measured by a reduction in edema, neuronal damage and astrogliotic tissue (34, 44, 45). Interestingly, the neuroprotection of AEA and arvanil was not only attenuated by $CB_1$ receptor blockade but also by a TRPV1 antagonist (45). The neuroprotective activity of 2-AG was also demonstrated in mice subjected to closed head injury (46). Increased levels of 2-AG were first observed as early as 1 h after injury, then they peaked at 4 h, but stayed above control levels for at least 24 h. Furthermore, when administered 15 min before contusion, 2-AG induced a dose-dependent reduction in brain edema monitored at 24 h, but, more importantly, it significantly enhanced clinical recovery up to 1 week after intervention. Hippocampal neuron loss was also reduced by 2-AG administration (46). The involvement of $CB_1$ receptors in neuroprotection is further supported by the increased severity of stroke in $CB_1$ receptor knockout mice, as assessed by increased mortality, infarct size and neurological deficits after transient focal cerebral ischemia and by enhanced neurotoxicity of NMDA in nonischemic mice (47, 48).

## Cannabinoid neuroprotection in Alzheimer's disease

Activated microglia are almost always present at the hallmark pathological feature of AD —the senile plaque— where they cluster (16, 49). They are believed to be central to the ongoing inflammatory process in the disease, and microglial activation is also a feature observed both in transgenic mice models of AD (50, 51) and following focal injection of Aβ into the brain (52). Furthermore, microglial activation results in neurodegeneration both *in vitro* and *in vivo* (53, 54). These observations further support the notion that limiting microglial activation and inflammation in AD may be considered of major therapeutic interest. Microglial cells as a target for cannabinoid therapy have the additional advantage of expressing not only $CB_1$ receptors, like the other types of neural cell, but also $CB_2$ receptors. Indeed, $CB_2$ receptors in brain may be uniquely expressed by microglia. One of the difficulties in introducing cannabinoids into the clinic is their psychoactivity, whether affective (euphoria), somatic (somnolence, motor incoordination), sensorial (altered temporal and spatial perception) or cognitive (memory lapses, confusion), which are all mediated by $CB_1$ activation. Therefore, we thought it would be interesting to investigate whether cannabinoids might have a selective effect on microglia by limiting their activation and subsequent neurotoxicity.

We first studied the localization, expression and function of CB receptors in AD postmortem brain tissue, with particular emphasis on any association with microglial cells and their activation (55), using immunohistochemical, pharmacological and biochemical techniques. Our results were in agreement with a recent work (56), that $CB_1$ and $CB_2$ receptors are expressed in senile plaques of AD brains along with markers of microglial activation. $CB_1$-positive neurons exist in high numbers in normal human brains, and we found a marked reduction of these neurons in AD frontal cortex, in particular in areas showing enhanced microglial activation. We demonstrated that $CB_1$ receptor protein expression was greatly reduced in AD and, more importantly, so was its function as assessed by G-protein coupling induced by the cannabinoid agonist WIN-55212-2. Since increased nitration is also evident in senile plaques of AD brain, we studied whether altered nitration of CB receptors might explain their reduced function. As anticipated, we observed increased $CB_1$ and $CB_2$ nitration, a chemical alteration that inactivates other proteins in AD brains (57).

Next we examined the possible protective effects of cannabinoids in rats injected intracerebroventricularly with Aβ for 7 days (20 mcg/rat/day) as a model of AD, since these animals show similar behavioral, biochemical and pathological alterations as patients with AD. Increased microglial activation was observed at 8 days after beginning Aβ administration and astrogliosis was observed at 15 days. A marked reduction in learning abil-

*Table II: Experimental models and treatment characteristics of cannabinoids as neuroprotectants against acute brain damage.*

| Model | Brain region | Drug/dose/time | Survival | References |
|---|---|---|---|---|
| Ouabain | Striatum/cortex | AEA, 10 mg/kg, –30 min | 7d | 34, 44 |
| | Striatum | AEA, 10 mg/kg, –30 min | 7d | 45 |
| | | Arvanil, 1 mg/kg | 7d | 45 |
| Head injury | Hippocampus | 2-AG, 0.1-10 mg/kg, +15 min | 1-7d | 46 |
| Ischemia | Hippocampus | WIN, 0.03-1 mg/kg, –40 min | 3d | 38 |
| | | WIN, 1 mg/kg, –30 to +30 min | 1d | |
| Asphyxia | Hippocampus | WIN, 0.1 mg/kg, +10 min | 7d | 43 |

Different experimental models of acute brain injury have been used. A single injection of cannabinoids was systemically administered to rodents at the doses and times indicated and their effects were studied at different survival times. AEA: anandamide; 2-AG: 2-arachinodoylglycerol; WIN: WIN-55212-2.

ity in a spatial navigation task (Morris water maze) was also seen at 2 months (Fig. 2). Moreover, in cerebral cortex there was a similar reduction to that found in AD patients in the expression of $CB_1$ receptor protein and in several neuronal markers. Furthermore, microglial activation, astrogliosis, cognitive impairment and loss of neuronal markers was prevented in rats that received cannabinoids together with Aβ (Fig. 2). Any changes indicative of adverse side effects of cannabinoids were carefully monitored, especially alterations in locomotor activity, including both horizontal and vertical activity and stereotypies, which were only present on the first day of cannabinoid injection but not on day 7 or 2 months later. Finally, immediately after treatment cessation the general hematological profiles of WIN-55212-2 treated rats were normal, as were the biochemical parameters and markers of tissue damage.

To gain insight into the mechanism of cannabinoid protection we studied the effects of cannabinoids on microglial activation and neurodegeneration induced by Aβ addition to cultures. As expected, cultured microglial cells expressed both $CB_1$ and $CB_2$ receptors. Aβ addition to microglia cultures increased mitochondrial respiration, changed their round appearance to a rod-like morphology and enhanced TNF-α release. Cannabinoids did not alter the basal values of these parameters but effectively counteracted Aβ-induced microglial activation. Interestingly, the effect of HU-210 was mimicked by WIN-55212-2, which is devoid of antioxidant capacity, and by the selective $CB_2$ agonist JWH-133. Aβ exerts both direct toxicity on neurons and indirect toxicity through microglia-mediated activation. Direct toxicity of high concentrations of Aβ on cultured neurons or astrocytes were unaffected by cannabinoids. We then performed experiments using microglia/neuron cocultures. To avoid direct toxicity, microglial cells seeded in inserts were treated with Aβ at nontoxic concentration for 4 h, allowing sufficient time for microglial activation, and then cultured them with neurons for another 20 h. In this paradigm, WIN-55212-2 and JWH-133 prevented Aβ toxicity. The neuroprotective effect of WIN-55212-2 was blocked by the selective $CB_1$ and $CB_2$ antagonists SR-141716 and SR-144528, while the effect of JWH-133 was only counteracted by the latter.

In summary, we have described neuroprotective effects of cannabinoids in models of AD *in vitro* and *in vivo* in which limiting microglial activation plays a crucial role. The fact that a $CB_2$ agonist is effective in this respect is of importance considering that the psychoactive effects of cannabinoids may cause concern when using them in a clinical setting.

## Future directions

Undoubtedly there are several issues that must be addressed in preclinical studies with cannabinoids before any clinical trials aimed at preventing or slowing the progress of AD can be performed. For instance, the route of administration and physicochemical characteristics of cannabinoids should be investigated and improved, the pharmacological selectivity of the drugs should be studied following long-term administration, the unwanted side effects should be monitored and the molecular and cellular mechanism of action should be further defined. Intracranial administration of drugs as has been done in the present work has very limited application in a clinical context. Therefore, systemic administration will be used in further studies, but this allows the drugs to reach the entire organism rather than just the brain. As already mentioned cannabinoid receptors are widely distributed in the periphery as well. In particular, the modulatory effects of cannabinoids on immune function may be a double edged sword (58) and should be taken into account. The bioavailability is high due to the lipophilicity of the compounds, although this property poses problems concerning route of administration. Indeed, the development of water-soluble cannabinoids is greatly needed and would allow their oral administration. One of the major concerns with mixed cannabinoid agonists may be their psychoactivity mediated by $CB_1$ receptor activation. This may be theoretically circumvented by using very low doses that may be neuroprotective with no accompanying psychoactivity, but this has not yet been investigated. In this respect it should be mentioned that acute treatment with cannabinoids has negative effects on learning and memory. This may have been the rationale for developing $CB_1$ antagonists as a pharmacological strategy for the treatment of AD. For example, one such $CB_1$ antagonist, AVE-1625, is undergoing phase II clinical trials in AD patients (4). However, the effects of cannabinoids on memory in some instances may have been overestimated. The disrupting effects of cannabinoids on working memory are dose-dependent and low doses have no effect. Moreover, mice lacking $CB_1$ receptors exhibited the same acquisition rate as wild-type mice in the Morris water maze in a fixed platform procedure, and only demonstrated significant deficits in a reversal task, pointing to a role of $CB_1$ in facilitating extinction or forgetting processes (59, 60). Nevertheless, it should be taken into account that subchronic cannabinoid exposure produced lasting memory impairment and increased anxiety in adolescent but not adult rats (61). In contrast, $CB_2$ agonists are devoid of psychoactive effects (62) and upon repeated intratumoral administration JWH-133 maintained its pharmacological selectivity. In any case, more behavioral studies with these compounds are needed.

The therapeutic potential of cannabinoids has been disregarded for a long time. This situation is rapidly changing given the very promising results obtained in recent studies both in animals and in humans. Undoubtedly, our increasing knowledge of the cannabinoid system and its pharmacology will result in the use of these compounds in the treatment of more human disorders. In the case of AD, cannabinoids that combine anti-inflammatory properties (58) with neuroprotective activity (63) may be suitable therapeutic agents.

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