

## NEUROPROTECTIVE, NON CBS-DEPENDANT ACTIVITIES OF PHYTOCANNABINOIDS

Capasso, A.; Saturnino, C.\*; Russo, L.; Rastrelli, L.

Department of Pharmacy, University of Salerno, 84084-Fisciano, Italy

[\\*saturnino@unisa.it](mailto:*saturnino@unisa.it)

### Abstract

The cannabinoids are found to have particular application as neuroprotectants because of their antioxidant properties. The antioxidative properties of cannabinoids suggest a therapeutic use as neuroprotective agents in limiting neurological damage. The antioxidative properties of cannabinoids suggest a therapeutic use as neuroprotective agents: these treatments should not only aim to alleviate specific symptoms but also attempt to delay/arrest disease progression and to repair the damaged structures. The studies reported in the present review support the view that the cannabinoid signalling system is a key modulatory element in the activity of the basal ganglia. This idea is supported by different anatomical, electrophysiological, pharmacological and biochemical data. Furthermore, these studies indicate that the cannabinoid system is impaired in different neurological disorders that directly or indirectly affect the basal ganglia, which supports the idea of developing novel pharmacotherapies with compounds that selectively target specific elements of the cannabinoid system.

**Keywords:** Cannabinoids; antioxidant; neuroprotection

## Introduction

The recreational use of Cannabis Sativa preparations is known to most people [1]. However, the medicinal use of Cannabis also has a millenarian history that has been re-examined only very recently [2]. As early as 2600 BC, the Chinese emperor Huang Ti advised taking Cannabis for the relief of cramps and rheumatic and menstrual pain [3]. This long history of Cannabis medical use has resulted in the development of pharmaceutical drugs, such as Dronabinol and Cesamet. These preparations is based on  $\Delta^9$ -tetrahydrocannabinol (THC, fig.1), which in 1964 was identified by Mechoulam and coworkers as the major psychoactive component of cannabis. They are prescribed in the United States as anti-emetic and appetite-stimulants to patients with cancer and AIDS. To date, some 60 plant terpenophenols more or less related to THC have been isolated and defined cannabinoids [4].  $\Delta^9$ -tetrahydrocannabinol, for its potency and abundance in cannabis, is the most important.

## Cannabinoid receptors

Thus far, two cannabinoid-specific receptors have been cloned and characterized from mammalian tissues, the seven transmembrane G protein-coupled cannabinoid receptors type 1 (CB1 receptor), [5] and type 2 (CB2 receptor) [6]. Whereas the CB1 receptor expression is abundant in the central nervous system, the CB2 receptor is almost exclusively expressed in the immune system. The CB1 receptor is also expressed in peripheral nerve terminals and various extraneuronal sites such as the testis, uterus, eye, vascular endothelial, spleen and adipocytes [7-10]. Pharmacological evidence exists for the presence of other cannabinoid receptors, which, however, have not yet been cloned [11].

CB1 and CB2 receptors share only 44% overall identity and 68% within the transmembrane domains. Both cannabinoid receptors are coupled to G proteins, mostly of the  $G_{i/o}$  type, through whose  $\alpha$  subunit they inhibit the activity of adenylate cyclases and stimulate mitogen-activated protein kinases. However, additional studies established that cannabinoid receptors were also coupled to ion channels, resultant in the inhibition of  $Ca^{2+}$  influx through N type calcium channels [12]. CB1 receptors are also implicated in activation of both phospholipase C (via the  $\beta\gamma$  subunits of the G protein) and PI-3-kinase. CB2 receptors, on the other hand, trigger a sustained activation of ceramide biosynthesis [13].

## The endocannabinoid system

Several endogenous fatty-acid ligands, known as endocannabinoids, have been identified as having activity at the cannabinoid receptor. The first to discovered, in 1992, was arachidonylethanolamide (anandamide, AEA) followed by 2-arachidonoylglycerol (2-AG). Both these compounds are derivatives of arachidonic acid conjugated with ethanolamine or glycerol and are able to bind to CB1 and CB2 receptors, although with differences in affinities and activation efficacies [8]. During the last few years, several other bioactive lipid mediators have described; they appear to be active, through CB1 and/or CB2 receptors and confer specific pharmacological effects in vivo. Specifically, the compounds are 2-arachidonoyl-glyceryl-ether (noladin ether), o-arachidonoyl-ethanolamine (virodhamine), N- arachidonoyl-dopamine, and possibly oleamide [14; 10; 15; 16] (fig. 2). Cannabinoid receptors, endocannabinoids and the whole apparatus appointed of their synthesis and degradation represent the elements of a novel endogenous signalling system (the endocannabinoid system) which is implicated in a overabundance of physiological functions [17; 18]. During the last few years a notable quantity of data has been reported to understand the biological roles of this system in more detail. In general, endocannabinoid system serves several functions under physiological conditions. In the CNS, endocannabinoids intervene in the regulation of cognitive functions and emotions in neuronal circuits of the cortex, hippocampus and amygdale and to the reinforcement of substances of abuse in the mesolimbic system [19].

Endocannabinoids also modulate the control of movement and posture [20], the regulation of pain perception [21] and cardiovascular [22], gastrointestinal [23], respiratory and reproductive functions. CB2 receptors, instead, are involved in cellular and particularly humoral immune response, with possible implications for (neuro)inflammation and chronic pain [25]. Apart from the possible physiological functions of the endocannabinoid system briefly described above, endocannabinoid signalling undergoes dramatic tissue and blood changes under pathological conditions. Higher endocannabinoid levels are found in the case of experimental models of neurodegenerative disease, like Parkinson's and Alzheimer's disease and amyotrophic lateral sclerosis, in gastrointestinal disorders like colon inflammation and in eating and metabolic disorders like anorexia nervosa, binge-eating disorders and obesity [26]. Finally, yet importantly, elevated levels of endocannabinoids

have been observed in several types of cancer like glioblastoma [27], meningioma [27], colon [28] and prostate [29] carcinoma, colon polyps [28] and pituitary adenoma [30] as compared to their normal counterparts, suggesting a function of the endocannabinoid as potential tumor growth inhibitors.

#### ***Anti-oxidative and neuroprotective actions of cannabinoids***

In the last years the number of studies related to anti-oxidative and neuroprotective actions of CBD are increased.

Hampson *et al.* [31] demonstrated that CBD reduced glutamate toxicity mediated by N-methyl-D-aspartate receptors (NMDAR), 2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl) propionic acid receptors (AMPA) or kainate receptors. The neuroprotection observed with cannabidiol was not affected by a cannabinoid receptor antagonist, indicating it is cannabinoid-receptor independent [31]. Previous studies had shown that glutamate toxicity may be prevented by antioxidants. In line with this, it was demonstrated that CBD can reduce hydroperoxide-induced oxidative damage as well as or better than other antioxidants. CBD was more protective against glutamate neurotoxicity than either ascorbate or  $\alpha$ -tocopherol, indicating that this drug is a potent antioxidant [31].

Another study [32] showed that the anti-oxidative action of CBD may induce the neuroprotection in animal models of Parkinson's disease (PD). Daily administration of CBD during 2 weeks may produce a significant waning in the magnitude of toxic effects caused by a unilateral injection of 6-hydroxydopamine into the medial forebrain bundle [32] probably due to receptor-independent actions. In this model of PD, CBD led to an up-regulation of mRNA levels of Cu/Zn-superoxide dismutase, a key enzyme in endogenous defense against oxidative stress. The conclusion was that the antioxidant properties of CBD can provide neuroprotection against the progressive degeneration of nigrostriatal dopaminergic neurons that occur in PD [33]. This study was confirmed by a study showing that CBD reduced the striatal atrophy caused by 3-nitropropionic acid, *in vivo*, through mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A<sub>2A</sub> receptors [34]. Also, the neuroprotective action of CBD in the human basal ganglia was supported by the strong relationship between N-acetylaspartate/total creatine ratio and CBD in the putamen/globus pallidum found in recreational cannabis users.

This could reflect an enhancement of neuronal and axonal integrity in these regions by CBD [35]. Given the above preclinical evidences, for the first time, a clinical study evaluated the efficacy, tolerability and safety of CBD in PD patients with psychotic symptoms [36]. In an open-label pilot study, six consecutive outpatients with the diagnosis of PD and who also had psychosis for at least 3 months, have received a flexible-dose regimen of CBD administration (starting with an oral dose of 150 mg/day) for four weeks, in addition to their usual therapy. The psychotic symptoms significantly decreased along the CBD treatment, and the scale used to follow up the PD course exhibited a significant decrease of the total score. These preliminary data suggest that CBD may have a beneficial action in PD [37]. The possible neuroprotective actions of CBD was also considered in Alzheimer's disease (AD). Alzheimer's disease is widely held to be associated with oxidative stress due, in part, to the membrane action of  $\beta$ -amyloid peptide aggregates. Iuvone *et al.* [38] studied the effect of cannabidiol, a major non-psychoactive component of the marijuana plant (*Cannabis sativa*) on  $\beta$ -amyloid peptide-induced toxicity in cultured rat pheocromocytoma PC12 cells. Following exposure of cells to  $\beta$ -amyloid peptide (1  $\mu$ g/mL), a marked reduction in cell survival was observed. This effect was associated with increased reactive oxygen species (ROS) production and lipid peroxidation, as well as caspase 3 (a key enzyme in the apoptosis cell-signalling cascade) appearance, DNA fragmentation and increased intracellular calcium. Treatment of the cells with cannabidiol ( $10^{-7}$ – $10^{-4}$ m) prior to  $\beta$ -amyloid peptide exposure significantly elevated cell survival while it decreased ROS production, lipid peroxidation, caspase 3 levels, DNA fragmentation and intracellular calcium. These results indicate that cannabidiol exerts a combination of neuroprotective, anti-oxidative and anti-apoptotic effects against  $\beta$ -amyloid peptide toxicity, and that inhibition of caspase 3 appearance from its inactive precursor, pro-caspase 3, by cannabidiol is involved in the signalling pathway for this neuroprotection. A possible anti-inflammatory action may be involved in this CBD effect, since CBD inhibited both nitrite production and nitric oxide synthase (iNOS) protein expression induced by beta-A [39]. These results of *in vitro* studies were confirmed *in vivo* with a mouse model of AD-related neuroinflammation. Mice were inoculated with human beta-A into the right dorsal hippocampus, and treated daily with vehicle or CBD (2.5 or 10 mg/kg, *i.p.*) for 7 days. In contrast to vehicle, CBD dose-dependent significantly inhibited

mRNA for glial fibrillary acidic protein and the protein expression in beta-A injected animals. Moreover, under the same experimental conditions, CBD impaired iNOS and IL-1beta protein expression, and the related NO and IL-1beta release [40]. The possibility of CBD inhibiting beta-A-induced neurodegeneration is very promising to AD prevention.

Recently it has been suggested that CBD may protect neurons against the multiple molecular and cellular factors involved in the different steps of the neurodegenerative process, which takes place during prion infection [41]. Prion diseases are transmissible neurodegenerative disorders characterized by the accumulation in the CNS of the protease-resistant prion protein, a structurally misfolded isoform of its physiological counterpart [41]. The anti-oxidative and anti-inflammatory properties of CBD have led to the research of its possible activity in preventing damage caused by cerebral ischemia. CBD (1.25-20 mg/kg) was administered to freely-moving gerbils 5 min after bilateral carotid-artery occlusion for 10 minutes. Seven days after the ischemia, CBD antagonized electroencephalographic flattening, showing a dose-dependent bell-shaped curve. The best neuroprotective effect was observed at 5 mg/kg. Histological examination showed the complete survival of CA1 neurons in CBD-treated gerbils [42]. A similar effect has been reported by another research group in mice, after middle cerebral artery occlusion; the neuroprotective action of CBD being unaffected by CB<sub>1</sub> receptor blockade [43]. The same research group has verified that this effect was inhibited by WAY100135, a serotonin 5-hydroxytryptamine 1A (5-HT<sub>1A</sub>) receptor antagonist, but not by capsazepine, a vanilloid receptor antagonist, suggesting that the neuroprotective effect of CBD may be due to the increase in cerebral blood flow mediated by the serotonergic 5-HT<sub>1A</sub> receptor [44]. Experimental evidence has suggested that beyond this action on the 5-HT<sub>1A</sub> receptor, the protective effect of CBD on ischemic injury is also secondary to its anti-inflammatory action [45]. In another study, the same research group reported that, while repeated treatment with delta9-THC leads to the development of tolerance for this neuroprotective effect, this phenomenon is not observed with CBD [46]. CBD has also been proven useful for possible complications of diabetes. The majority of diabetic complications are associated with pathophysiological alterations in the vasculature. Microvascular complications involve retinopathy and nephropathy while the

atherosclerosis is the most common macrovascular complication of diabetes. The protective effects of CBD were studied in experimental diabetes induced by streptozotocin in rats. CBD treatment prevented retinal cell death and vascular hyperpermeability in the diabetic retina. In addition, it significantly reduced oxidative stress, decreased the levels of TNF- $\alpha$ , vascular endothelial growth factor, and intercellular adhesion-molecule [47]. It has also been suggested that CBD has significant therapeutic benefits against other diabetic complications and atherosclerosis, since it attenuated several effects of high glucose, including the disruption of the endothelial function [48].

#### ***Recent studies on mechanism of antioxidant action of cannabinoids***

Dexanabinol, HU-211, a synthetic cannabinoid devoid of psychotropic effects, improves neurological outcome in models of brain trauma, ischemia and meningitis. Also, HU-211 was found to inhibit brain tumor necrosis factor (TNF $\alpha$ ) production after head injury. Gallily *et al.* [49] demonstrated the ability of HU-211 to suppress TNF $\alpha$  production and to rescue mice and rats from endotoxic shock after LPS (*Escherichia coli* 055:B5) inoculation. In BALB/c mice, a dose of 10 mg/kg LPS, injected *i.p.*, caused 57% and 100% mortality, at 24 and 48 hr, respectively. HU-211, administered *i.p.* 30 min before lipopolysaccharide (LPS), reduced lethality to 9 and 67% at these time points ( $P < .05$ ). When co-injected with D-galactoseamine (*i.p.*), LPS was 100% lethal within 24 hr, whereas eight hourly injections of HU-211 caused mortality of C57BL/6 mice to drop to 10% ( $P < .001$ ). Administration of LPS to Sprague-Dawley rats resulted in a 30% reduction in the mean arterial blood pressure within 30 min, which persisted for 3 hr. HU-211, given 2 to 3 min before LPS, completely abolished the typical hypotensive response. Furthermore, the drug also markedly suppressed *in vitro* TNF $\alpha$  production and nitric oxide generation (by >90%) by both murine peritoneal macrophages and rat alveolar macrophage cell line exposed to LPS. HU-211 may, therefore, have therapeutic implications in the treatment of TNF $\alpha$ -mediated pathologies [49]. The results of the present *in vivo* studies, in two experimental models using two species, suggest that HU-211 may have important clinical implications. It is noteworthy that dexanabinol, HU-211, was tested in human volunteers in phase I clinical trial, and is now under phase II clinical trial for severe head injury. Thus, this novel drug appears to be a promising candidate for the treatment in the nontreatable and

devastating TNF $\alpha$ -mediated diseases. Interleukin-1 receptor antagonist (IL-1ra) is an important anti-inflammatory cytokine that blocks all known actions of IL-1 and markedly protects against experimentally induced ischemic, excitotoxic, and traumatic brain insults. Cannabinoids (CBs) also exert potent anti-inflammatory and neuroprotective effects, but the mechanisms of their actions are unknown. Molina-Holdago *et al.* [50] tested the hypothesis that the actions of CBs were mediated by endogenous IL-1ra. They reported for the first time that both CB<sub>1</sub> and CB<sub>2</sub> receptors modulate release of endogenous IL-1ra from primary cultured glial cells [50]. Activation of CB<sub>1</sub> or CB<sub>2</sub> receptors increased lipopolysaccharide-induced IL-1ra release, and specific CB<sub>1</sub> or CB<sub>2</sub> antagonists blocked lipopolysaccharide-induced production of IL-1ra from glial cells. Comparison of neuronal cultures from wild-type mice and mice lacking IL-1ra (knock-out) indicates that endogenous IL-1ra is essential for the neuro-protective effects of CBs against excessive activation of glutamate receptors (excitotoxicity) in response to S-AMPA or NMDA. Similarly, analysis of mixed glial cultures from IL-1ra knock-out mice indicates that endogenous IL-1ra is required for the CB-induced inhibition of nitric oxide production in response to bacterial lipopolysaccharide [50]. These data suggest a novel neuroprotective mechanism of action for CBs in response to inflammatory or excitotoxic insults that is mediated by both CB<sub>1</sub> and CB<sub>2</sub> receptor-dependent pathways. In summary, the results presented here support our hypothesis that endogenous IL-1ra mediates the neuroprotective and anti-inflammatory actions of CBs in primary neurons and glia. These effects appear to be mediated by both CB<sub>1</sub> and CB<sub>2</sub> receptors. CB-induced IL-1ra release may negatively regulate IL-1 $\beta$  actions in the brain, via IL-1ra blocking the IL-1 receptor (IL-1RI), after inflammatory or excitotoxic insults. It is tempting to speculate therefore that the neuroprotective and anti-inflammatory actions of CBs depend in part on modification of the balance between proinflammatory and anti-inflammatory cytokines. These findings have important implications for our understanding of the mechanisms of action of CBs in diverse CNS disorders and for the development of new neuroprotective therapies. CB<sub>1</sub> cannabinoid receptors (CB<sub>1</sub>Rs) are involved in protecting the brain from ischemia and related disorders. However, the underlying protective mechanisms are incompletely understood. Kim *et al.* [51] investigated the effect of CB<sub>1</sub>R activation on

oxidative injury, which has been implicated in neuronal death after cerebral ischemia and neurodegenerative disorders, in mouse cortical neuron cultures. The CB<sub>1</sub>R agonist Win 55212-2 [R-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate] reduced neuronal death, measured by lactate dehydrogenase release, in cultures treated with 50 microM FeCl<sub>2</sub>, and its protective effect was attenuated by the CB<sub>1</sub>R antagonist SR141716A [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride]. The endocannabinoid anandamide reproduced the effect of Win 55212-2, as did the antioxidant 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). Neuronal injury was more severe after *in vitro* or *in vivo* administration of FeCl<sub>2</sub> to CB<sub>1</sub>R-knockout compared with wild-type mice [51]. Win 55212-2 reduced the formation of reactive oxidative species in cortical neuron cultures treated with FeCl<sub>2</sub>, consistent with an antioxidant action [41]. Pertussis toxin reduced CB<sub>1</sub>R-mediated protection, which points to a protective mechanism that involves signaling through G(i/o) proteins. Since CB<sub>1</sub>R-activated G protein signaling inhibits protein kinase A but activates phosphatidylinositol 3-kinase (PI3K), Kim *et al.* [51] tested the involvement of these pathways in CB<sub>1</sub>R-mediated neuroprotection. Dibutyryl-cyclic adenosine monophosphate (dbcAMP) blocked protection by Win 55212-2, whereas the PI3K inhibitor wortmannin did not, and the effect of dbcAMP was inhibited by the protein kinase A inhibitor H89 [N-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide] (> or =10 nM). CB<sub>1</sub>R-induced, SR141716A-, pertussis toxin-, and dbcAMP-sensitive protection was also observed for two other oxidative insults, exposure to H<sub>2</sub>O<sub>2</sub> or buthionine sulfoximine. Therefore, receptor-stimulated inhibition of protein kinase A seems to be required for the neuroprotective effect of CB<sub>1</sub>R activation against oxidative neuronal injury. These precedents will be helpful in guiding future studies on mechanisms of cannabinoid neuroprotection. Alzheimer's disease (AD) is characterized by enhanced beta-amyloid peptide (betaA) deposition along with glial activation in senile plaques, selective neuronal loss, and cognitive deficits. Cannabinoids are neuroprotective agents against excitotoxicity *in vitro* and acute brain damage *in vivo* [52]. Ramirez *et al.* [52] have studied the localization, expression, and function of cannabinoid receptors in AD and the

possible protective role of cannabinoids after betaA treatment, both in vivo and in vitro. They showed that senile plaques in AD patients express cannabinoid receptors CB1 and CB2, together with markers of microglial activation, and that CB1-positive neurons, present in high numbers in control cases, are greatly reduced in areas of microglial activation [52]. In pharmacological experiments, they found that G-protein coupling and CB1 receptor protein expression are markedly decreased in AD brains. Additionally, in AD brains, protein nitration is increased, and, more specifically, CB1 and CB2 proteins show enhanced nitration [52]. Intracerebroventricular administration of the synthetic cannabinoid WIN55,212-2 to rats prevent betaA-induced microglial activation, cognitive impairment, and loss of neuronal markers [52]. Cannabinoids (HU-210, WIN55,212-2, and JWH-133) block betaA-induced activation of cultured microglial cells, as judged by mitochondrial activity, cell morphology, and tumor necrosis factor-alpha release; these effects are independent of the antioxidant action of cannabinoid compounds and are also exerted by a CB2-selective agonist [52]. Moreover, cannabinoids abrogate microglia-mediated neurotoxicity after betaA addition to rat cortical cocultures. These results indicate that cannabinoid receptors are important in the pathology of AD and that cannabinoids succeed in preventing the neurodegenerative process occurring in the disease. The neuroprotective effects of Delta(9)-tetrahydrocannabinol (THC) were examined by Chen *et al.* [53] using an in vitro model in which the AF5 CNS cell line was exposed to toxic levels of N-methyl-d-aspartate (NMDA), an agonist of the NMDA glutamate receptor. NMDA toxicity was reduced by THC, but not by the more specific cannabinoid receptor agonist, WIN55,212-2. Addition of dibutyryl cAMP (dbcAMP) to the culture medium did not alter the neuroprotective effect of THC and did not unmask a neuroprotective effect of WIN55,212-2. The cannabinoid antagonist SR141716A did not inhibit the neuroprotection induced by THC or alter the response to WIN55,212-2, even in the presence of dbcAMP, indicating that the neuroprotective effect of THC was cannabinoid receptor-independent. On the other hand, both THC and WIN55,212-2 produced cellular toxicology at higher dosages, an effect which was blocked in part by SR141716A. Capsaicin, an antioxidant and vanilloid receptor agonist, also produced a protective effect against NMDA toxicology [53]. The protective effect of capsaicin

was blocked by co-application of ruthenium red, but was not blocked by the specific vanilloid receptor antagonist capsazepine, and the transient receptor potential vanilloid type 1 (TRPV1) and ANKTM1 transcripts were not detected in AF5 cells. Thus, the neuroprotective effects of THC and capsaicin did not appear to be mediated by TRP ion channel family receptors. The antioxidant alpha-tocopherol prevented neurotoxicity in a dose-dependent manner. Therefore, THC may function as an antioxidant to increase cell survival in NMDA-induced neurotoxicity in the AF5 cell model, while higher dosages produce toxicity mediated by CB1 receptor stimulation [53].

Binge alcohol consumption in the rat induces substantial neurodegeneration in the hippocampus and entorhinal cortex. Oxidative stress and cytotoxic edema have both been shown to be involved in such neurotoxicity, whereas N-methyl-d-aspartate (NMDA) receptor activity has been implicated in alcohol withdrawal and excitotoxic injury. Because the nonpsychoactive cannabinoid cannabidiol (CBD) was previously shown in vitro to prevent glutamate toxicity through its ability to reduce oxidative stress, Hamelink *et al.* [54] evaluated CBD as a neuroprotectant in a rat binge ethanol model. When administered concurrently with binge ethanol exposure, CBD protected against hippocampal and entorhinal cortical neurodegeneration in a dose-dependent manner. Similarly, the common antioxidants butylated hydroxytoluene and alpha-tocopherol also afforded significant protection. In contrast, the NMDA receptor antagonists dizocilpine (MK-801) and memantine did not prevent cell death [54]. Of the diuretics tested, furosemide was protective, whereas the other two anion exchanger inhibitors, L-644,711 [(R)-(+)-(5,6-dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-propyl-1H-fluoren-7-yl)oxy acetic acid] and bumetanide, were ineffective [54]. In vitro comparison of these diuretics indicated that furosemide is also a potent antioxidant, whereas the nonprotective diuretics are not. The lack of efficacy of L-644,711 and bumetanide suggests that the antioxidant rather than the diuretic properties of furosemide contribute most critically to its efficacy in reversing ethanol-induced neurotoxicity in vitro, in our model. This study provides the first demonstration of CBD as an in vivo neuroprotectant and shows the efficacy of lipophilic antioxidants in preventing binge ethanol-induced brain injury [54]. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive motor neuron loss, paralysis and death within 2-5 years of diagnosis. Currently, no effective

pharmacological agents exist for the treatment of this devastating disease. Neuroinflammation may accelerate the progression of ALS. Cannabinoids produce anti-inflammatory actions via cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), and delay the progression of neuroinflammatory diseases [55]. Additionally, CB2 receptors, which normally exist primarily in the periphery, are dramatically up-regulated in inflamed neural tissues associated with CNS disorders. In G93A-SOD1 mutant mice, the most well-characterized animal model of ALS, endogenous cannabinoids are elevated in spinal cords of symptomatic mice [55]. Furthermore, treatment with non-selective cannabinoid partial agonists prior to, or upon, symptom appearance minimally delays disease onset and prolongs survival through undefined mechanisms. Shoemaker *et al.* [55] demonstrated that mRNA, receptor binding and function of CB2, but not CB1, receptors are dramatically and selectively up-regulated in spinal cords of G93A-SOD1 mice in a temporal pattern paralleling disease progression. More importantly, daily injections of the selective CB2 agonist AM-1241, initiated at symptom onset, increase the survival interval after disease onset by 56% [55]. Therefore, CB2 agonists may slow motor neuron degeneration and preserve motor function, and represent a novel therapeutic modality for treatment of ALS.

Impaired endothelial activity and/or cell death play a critical role in the development of vascular dysfunction associated with congestive heart failure, diabetic complications, hypertension, coronary artery disease and atherosclerosis. Increasing evidence suggests that cannabinoid 1 (CB1) receptor inhibition is beneficial in atherosclerosis and cardiovascular inflammation both in experimental models, as well as in humans. Rajesh *et al.* [56] investigated the effects of CB1 receptor activation with the endocannabinoid anandamide (AEA) or synthetic agonist HU210 on cell death and interrelated signal transduction pathways in human primary coronary artery endothelial cells (HCAECs). In HCAECs expressing CB1 receptors (demonstrated by Western immunoblot and flow cytometry) AEA (5-15  $\mu\text{M}$ ) or HU210 (30-1000 nM) triggered concentration- and time-dependent activation of p38 and c-Jun NH(2)-terminal protein kinase (JNK)-mitogen-activated protein kinases (MAPKs), cell death and ROS generation [56]. The AEA- or HU210-induced cell death and MAPK activation were attenuated by CB1 antagonists [SR141716 (rimonabant) and AM281], inhibitors of p38 and

JNK-MAPKs or the antioxidant N-acetylcysteine. N-acetylcysteine alone prevented AEA- or HU210-induced ROS generation, but only partially attenuated MAPK activation and cell death. In contrast, in combination with CB1 antagonists, N-acetylcysteine completely prevented these effects [56]. CB1 receptor activation in endothelial cells may amplify the ROS-MAPK activation-cell death pathway in pathological conditions when the endocannabinoid synthetic or metabolic pathways are dysregulated by excessive inflammation and/or oxidative/nitrosative stress, thereby contributing to the development of endothelial dysfunction and pathophysiology of multiple cardiovascular diseases [56].

### Conclusions

In conclusion, the present paper showed that cannabinoids possess antioxidant and neuroprotective effects. In addition to directly salvaging neurons affected by several disorders, cannabinoids also have anti-inflammatory effects and promote the birth of new neurons (neurogenesis) in the adult brain, either of which may contribute to improving neurological outcome. The antioxidative properties of cannabinoids suggest a therapeutic use as neuroprotective agents, and the particular properties of cannabidiol make it a good candidate for such development.

Neuroprotective activities of endocannabinoids appear to be CB1-mediated thus providing antioxidant protection. Therefore, the use of antioxidant cannabinoids could provide promising avenues for the therapeutic targeting of different aspects of neurodegenerative diseases, by stimulating a self-protective endogenous system of the brain and by counteracting oxidative stress.

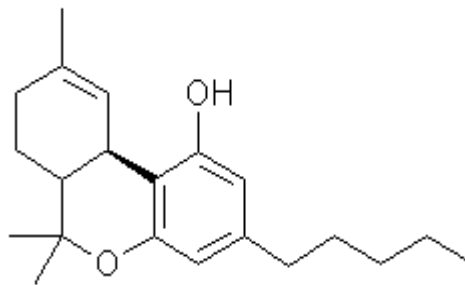
### References

1. Adams, I.B., Martin, B.R., Cannabis: pharmacology and toxicology in animals and humans. *Addiction* 1996; 91: 1585-1614.
2. Williamson, E.M., Evans, F.J., Cannabinoids in clinical practice. *Drugs* 2000; 60: 1303-1314.
3. Mechoulam, R., Cannabis as Therapeutic Agent (ed. Mechoulam, R.) CRC Press Boca Raton 1986; 1-19.
4. Mechoulam, R., Gaoni, Y., Recent advances in the chemistry of hashish. *Fortschr Chem Org Naturst* 1967; 25: 175-213.
5. Matsuda, L.A., Lolait, S.J., Brownstein, M.J., *et al.*, Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990; 346: 561-564.
6. Munro, S., Thomas, K.L., Abu-Shaar, M., Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993; 365: 61-65.
7. Howlett, A.C., Cannabinoid receptor signalling. *Handb Exp Pharmacol* 2005; 168: 53-79.
8. Howlett, A.C., The cannabinoid receptors. *Prostaglandins*

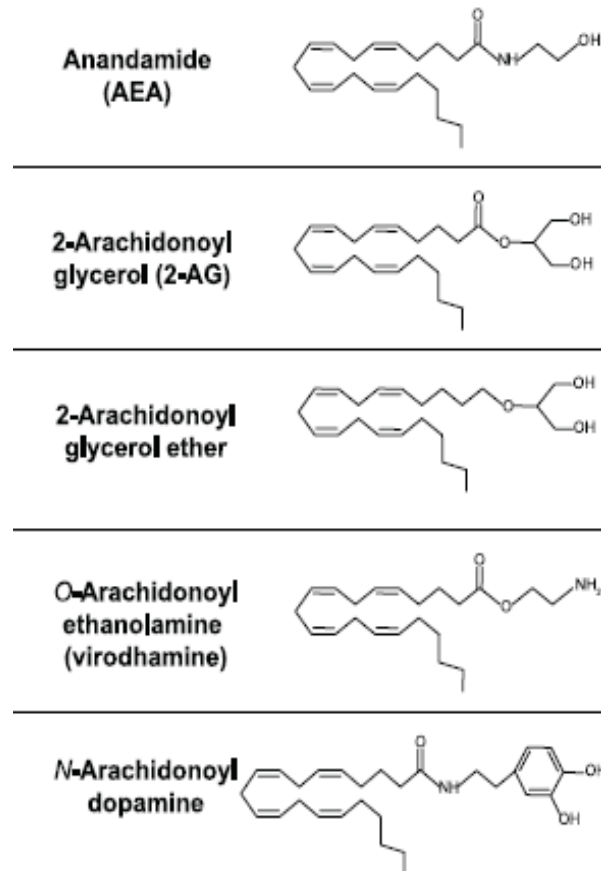
- Other Lipid Mediat 2002; 69: 619-631.
9. Herkenham, M., Lynn, A.B., Johnson, M.R., et al. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* 1991; 11: 563-583.
  10. Porter, A.C., Felder, C.C., The endocannabinoid nervous system. Unique opportunities for therapeutic intervention. *Pharmacol Ther* 2001; 90: 45-60.
  11. Begg, M., Pacher, P., Batkai, S., et al. Evidence for novel cannabinoid receptors. *Pharmacol Ther* 2005; 106: 133-145.
  12. Rodriguez De Fonseca, F., Del Arco, I., Bermudez-Silva, F.J., et al. The endocannabinoid system: physiology and pharmacology. *Alcohol Alcoholism* 2005; 40: 2-14.
  13. Howlett, A.C., Breivogel, C.S., Childers, S.R., et al. Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* 2004; 47: 345-358.
  14. Hanus, L., Abu-Lafi, S., Frède, E., et al. 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci USA* 2001; 98: 3662-3665.
  15. Huang, S.M., Bisogno, T., Trevisani, M., et al., An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci USA* 2002; 99: 8400-8405.
  16. Leggett, J.D., Aspley, S., Beckett, S.R., et al., Oleamide is a selective endogenous agonist of rat and human CB1 cannabinoid receptors. *Br J Pharmacol* 2004; 141:253-262
  17. Piomelli, D., The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* 2003;4:873-884
  18. De Petrocellis, L., Cascio, M.G., Di Marzo, V., The endocannabinoid system: a general view and latest additions. *Br J Pharmacol* 2004;141:765-774
  19. Gerdeman, G.L., Partridge, J., Lupica, C.R., et al., It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci* 2003; 26: 184-192.
  20. Van der Stelt, M., Di Marzo, V., The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. *Eur J Pharmacol* 2003; 480: 133-150.
  21. Iversen, L., Chapman, V., Cannabinoids: a real prospect for pain relief? *Curr Opin. Pharmacol* 2002; 2 :50-55.
  22. Randall, M.D., Harris, D., Kendall, D.A., et al., Cardiovascular effects of cannabinoids. *Pharmacol Ther* 2002; 95: 191-202.
  23. Di Carlo, G., Izzo, A.A., Cannabinoids for gastrointestinal diseases: potential therapeutic applications. *Expert Opin Investig Drugs* 2003; 12: 39-49.
  24. Wenger, T., Maccarrone, M., Effect of cannabinoids on hypothalamic and reproductive function. *Handb Exp Pharmacol* 2005; 168: 555-572.
  25. Klein, T.W., Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol* 2005; 5: 400-411.
  26. Di Marzo, V., Petrosino, S., Endocannabinoids and the regulation of their levels in health and disease. *Curr Opin Lipidol* 2007; 18: 129-140.
  27. Petersen G, Moesgaard B, Schmid PC, et al. Endocannabinoid metabolism in human glioblastomas and meningiomas compared to human non-tumor brain tissue. *J Neurochem* 2005; 93: 299-309.
  28. Ligresti, A., Bisogno, T., Matias, I., et al. Possible endocannabinoid control of colorectal cancer growth. *Gastroenterology* 2003; 125: 677-687.
  29. Schmid, P.C., Wold, L.E., Krebsbach, R.J., et al., Anandamide and other N-acyl ethanolamines in human tumors. *Lipids* 2002; 37: 907-912.
  30. Pagotto, U., Marsicano, G., Fezza, F., et al. Normal human pituitary gland and pituitary adenomas express cannabinoid receptor type 1 and synthesize endogenous cannabinoids: first evidence for a direct role of cannabinoids on hormone modulation at the human pituitary level. *J Clin Endocrinol Metab* 2001; 86: 2687-2696.
  31. Hampson, A.J., Grimaldi, M., Axelrod, Wink, D., Cannabidiol and delta 9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci USA*. 1998;95(14):8268-73.
  32. Lastres-Becker, I., Molina-Holgado, F., Ramos, J.A., et al., Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: relevance to Parkinson's disease. *Neurobiol Dis*. 2005;19(1-2):96-107.
  33. Garcia-Arencibia, M., Gonzalez, S., de Lago, E., et al., Evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson's disease: importance of antioxidant and cannabinoid receptor-independent properties. *Brain Res* 2007;1134(1):162-70.
  34. Sagredo, O., Ramos, J.A., Decio, A., et al., Cannabidiol reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A receptors. *Eur J Neurosci*. 2007;26(4):843-51.
  35. Hermann, D., Sartorius, A., Welzel, H., et al., Dorsolateral prefrontal cortex N-acetylaspartate/total creatine (NAA/tCr) loss in male recreational cannabis users. *Biol Psychiatry*. 2007;61(11):1281-9.
  36. Zuardi, A.W., Crippa, J.A.S., Hallak, J.E.C., et al., Cannabidiol for the treatment of psychosis in Parkinson's disease: possible mechanisms of actions. *J Psychopharmacology*. 2009 Nov;23(8):979-83.
  37. Iuvone, T., Esposito, G., Esposito, R., et al., Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on beta-amyloid-induced toxicity in PC12 cells. *J Neurochem* 2004;89(1):134-41.
  38. Esposito, G., De Filippis, D., Carnuccio, R., et al., The marijuana component cannabidiol inhibits beta-amyloid-induced tau protein hyperphosphorylation through Wnt/beta-catenin pathway rescue in PC12 cells. *J Mol Med* 2006;84(3):253-8.
  39. Esposito, G., De Filippis, D., Maiuri, M.C., et al., Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in beta-amyloid stimulated PC12 neurons through p38 MAP kinase and NF-kappaB involvement. *Neurosci Lett* 2006;399(1-2):91-5.
  40. Esposito, G., Scuderi, C., Savani, C., et al., Cannabidiol in vivo blunts beta-amyloid induced neuroinflammation by suppressing IL-1beta and iNOS expression. *Br J Pharmacol*. 2007;151(8):1272-9.
  41. Dirikoc, S., Priola, S.A., Marella, M., et al., Nonpsychoactive cannabidiol prevents prion accumulation and protects neurons against prion toxicity. *J Neurosci*. 2007;27(36):9537-44.
  42. Dirikoc, D., Pegorini, S., Arcidiacono, M.V., et al., Post-ischemic treatment with cannabidiol prevents electroencephalographic flattening, hyperlocomotion and neuronal injury in gerbils. *Neurosci Lett*. 2003;346(1-2):61-4.
  43. Hayakawa, K., Mishima, K., Abe, K., et al., Cannabidiol prevents infarction via the non-CB1 cannabinoid receptor mechanism. *Neuroreport*. 2004;15(15):2381-5.
  44. Mishima, K., Hayakawa, K., Abe, K., et al., Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. *Stroke*. 2005;36(5):1077-82.



45. Hayakawa, K., Mishima, K., Nozako, M., et al., Delayed treatment with cannabidiol has a cerebroprotective action via a cannabinoid receptor-independent myeloperoxidase-inhibiting mechanism. *J Neurochem* 2007;102(5):1488-96.
46. Hayakawa, K., Mishima, K., Nozako, M., et al., Repeated treatment with cannabidiol but not Delta9-tetrahydrocannabinol has a neuroprotective effect without the development of tolerance. *Neuropharmacology* 2007;52(4):1079-87.
47. El-Remessy, A.B., Al-Shabrawey, M., Khalifa, Y., et al., Neuroprotective and blood-retinal barrier-preserving effects of cannabidiol in experimental diabetes. *Am J Pathol* 2006;168(1):235-44.
48. Rajesh, M., Mukhopadhyay, P., Bátkai, S., et al., Cannabidiol attenuates high glucose-induced endothelial cell inflammatory response and barrier disruption. *Am J Physiol Heart Circ. Physiol* 2007;293(1):H610-9.
49. Gallily R, Yamin A, Waksman Y, Ovadia H, Weidenfeld J, Bar-Joseph A, Biegon A, Mechoulam R, Shohami E. Protection against septic shock and suppression of tumor necrosis factor alpha and nitric oxide production by dexanabinol (HU-211), a nonpsychotropic cannabinoid. *J Pharmacol Exp Ther.* 1997;283(2):918-24.
50. Molina-Holgado, F., Pinteaux, E., Moore, J.D., et al., Endogenous interleukin-1 receptor antagonist mediates anti-inflammatory and neuroprotective actions of cannabinoids in neurons and glia. *J Neurosci.* 2003;23(16):6470-4.
51. Kim, S.H., Won, S.J., Mao, X.O., et al., Greenberg DA. Involvement of protein kinase A in cannabinoid receptor-mediated protection from oxidative neuronal injury. *J Pharmacol Exp Ther* 2005;313(1):88-94.
52. Ramírez, B.G., Blázquez, C., Gómez del Pulgar, T., et al., Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci.* 2005 Feb 23;25(8):1904-13.
53. Chen, J., Lee, C.T., Errico, S., et al., Protective effects of Delta(9)-tetrahydrocannabinol against N-methyl-D-aspartate-induced AF5 cell death. *Brain Res Mol Brain Res.* 2005;134(2):215-25.
54. Hamelink, C., Hampson, A., Wink, D.A., et al., Comparison of cannabidiol, antioxidants, and diuretics in reversing binge ethanol-induced neurotoxicity. *J Pharmacol Exp Ther* 2005;314(2):780-8.
55. Shoemaker, J.L., Seely, K.A., Reed, R.L., et al., The CB2 cannabinoid agonist AM-1241 prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis when initiated at symptom onset. *J Neurochem* 2007 Apr;101(1):87-98.
56. Rajesh, M., Mukhopadhyay, P., Haskó, G., et al., Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. *Br J Pharmacol* 2010;160(3):688-700



**Figure 1.** Chemical structure of  $\Delta^9$ -tetrahydrocannabinol



**Figure 2.** Chemical structure of endogenous cannabinoid