The Endocannabinoid System in Amyotrophic Lateral Sclerosis

Lynsey G. Bilsland¹ and Linda Greensmith²,*

¹Molecular NeuroPathobiology, Cancer Research UK, 44 Lincoln’s Inn Fields, London and ²Institute of Neurology, University College London, Queen Square, London, UK

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative condition characterised by the selective loss of motor neurons from the spinal cord, brainstem and motor cortex. Although the pathogenic mechanisms that underlie ALS are not yet fully understood, there is significant evidence that several neurotoxic mechanisms including excitotoxicity, inflammation and oxidative stress, all contribute to disease pathogenesis. Furthermore, recent results have established that although primarily a motor neuron specific disorder, ALS is not cell-autonomous and non-neuronal cells including astroglia and microglia play a critical role in mechanism of disease. Currently the only licensed therapy available for the treatment of ALS is the anti-glutamatergic agent Riluzole, which has limited therapeutic effects. However, there is increasing evidence that cannabinoids and manipulation of the endocannabinoid system may have therapeutic value in ALS, in addition to other neurodegenerative conditions. Cannabinoids exert anti-glutamatergic and anti-inflammatory actions through activation of the CB1 and CB2 receptors, respectively. Activation of CB2 receptors may therefore inhibit glutamate release from presynaptic nerve terminals and reduce the postsynaptic calcium influx in response to glutamate receptor stimulation. Meanwhile, CB2 receptors may influence inflammation, whereby receptor activation reduces microglial activation, resulting in a decrease in microglial secretion of neurotoxic mediators. Finally, cannabinoid agents may also exert anti-oxidant actions by a receptor-independent mechanism. Therefore the ability of cannabinoids to target multiple neurotoxic pathways in different cell populations may increase their therapeutic potential in the treatment of ALS. Recent studies investigating this potential in models of ALS, in particular those that focus on strategies that activate CB2 receptors, are discussed in this review.

Key Words: Neurodegeneration, excitotoxicity, inflammation, oxidative stress, SOD1, neuroprotection, motor neuron, therapy.

INTRODUCTION

Originally described in 1869 by the French physician Jean-Martin Charcot, Amyotrophic Lateral Sclerosis (ALS) is the most common adult motor neuron disease in humans. ALS is characterised by the progressive degeneration of lower motor neurons in the spinal cord and brainstem, and the large pyramidal neurons in the motor cortex and associated corticospinal tracts. ALS is predominantly a sporadic disorder, although approximately 10% of cases have a familial history consistent with Mendelian inheritance. Following the discovery that a subset (approximately 20%) of familial ALS is caused by mutations in the Cu/Zn superoxide dismutase (SOD1) enzyme [1], transgenic mice expressing various mutant human SOD1 genes have been generated and have been widely used as a model for the disease. These transgenic mice develop a motor neuron disease with many of the pathological features seen in humans including neurofilament inclusions, ubiquitinylated aggregates and selective motor neuron loss, accompanied by severe muscle paralysis and premature death. These mice therefore providing a valuable research tool for the study of ALS pathogenesis and preclinical testing of potential therapies (reviewed in [2]).

Extensive research using SOD1 mice, in combination with analysis of post-mortem tissue from ALS patients has provided insight into the neurotoxic mechanisms that are involved in ALS. There is now strong evidence that implicates glutamate excitotoxicity, inflammation and oxidative stress, amongst other mechanisms, in ALS pathogenesis (reviewed in [2]). In this Review, we will discuss the evidence for the involvement of these mechanisms in ALS pathogenesis and then summarise the actions of the endocannabinoid system in targeting these mechanisms (see Fig. 1), in order to assess the therapeutic potential of modulation of the endocannabinoid system as a strategy for the treatment of ALS. A summary of the various studies in which the endocannabinoid system has been manipulated in models of ALS is shown in Table 1.

TARGETING OF GLUTAMATE EXCITOTOXICITY BY THE ENDOCANNABINOID SYSTEM IN ALS

Glutamate is the most abundant excitatory neurotransmitter in the CNS [3]. However, there is substantial evidence to suggest that over-activation of glutamate receptors can result in neuronal damage, a process termed ‘excitotoxicity’. Elevated extracellular glutamate, either due to an increase in release or a reduction in uptake, can activate glutamate receptors on the postsynaptic cell, thus enhancing calcium influx. Excessive postsynaptic calcium can subsequently activate neurotoxic cascades such as activation of calpains, endonucleases and phospholipases, ultimately leading to neuronal death [4].

Motor neurons receive glutamatergic inputs from the descending corticospinal tracts, from collaterals of the Aα fibres innervating muscle fibres and Golgi tendon organs and
from excitatory interneurons in the spinal cord [3]. Therefore, abnormalities in glutamate neurotransmission may have a particularly significant impact on motor neurons and may thereby contribute to motor neuron degeneration in ALS. Indeed, an elevation in cerebrospinal fluid glutamate levels occurs in approximately 40% of sporadic ALS patients [5, 6] and transcranial magnetic stimulation studies in ALS patients reveal that cortical motor neurons are hyperexcitable [7]. Furthermore, it has been reported that there is an increase in firing frequency and a reduction in action potential duration in embryonic motor neurons in vitro, which further indicates that there may be an increase in motor neuron excitability in ALS [8, 9].

In addition to an increase in motor neuron excitability, a reduction in glutamate uptake may also contribute to excitotoxicity in ALS. Reduced expression of the main excitatory amino acid transporter-2 (EAAT-2, also known in mice as glutamate transporter-1, GLT-1) and a decrease in glutamate transport has been found in 60-70% of sporadic ALS post-mortem spinal cord and motor cortex tissue [10, 11]. A mutation in EAAT-2 has also been identified in a sporadic ALS patient and was associated with a reduction in glutamate uptake [12]. Similarly, glutamate uptake is diminished in symptomatic SOD1 mice, and this is reflected in a reduction in EAAT2 (GLT-1) expression [13-15]. Recently, mutant SOD1 has been shown to have the ability to induce caspase-3 mediated cleavage of EAAT-2 (GLT-1) [16]. Indeed, an inactive, truncated form of EAAT2 (GLT-1) is found in spinal cords of presymptomatic SOD1 mice [16]. This fragment is sumoylated and accumulates in promyelocytic leukemia
nuclear bodies, which may interfere with their normal regulatory role in gene transcription [17]. In vitro, in organotypic spinal cord cultures, chronic inhibition of glutamate transport induces selective motor neuron degeneration [18], whereas restoration of EAAT2 (GLT-1) activity either by pharmacological enhancement [19] or by the introduction of glial progenitor cells over-expressing EAAT2 (GLT-1), significantly increases motor neuron survival [20].

Glutamate-mediated excitotoxicity in ALS is thought to result from activation of calcium permeable AMPA/KA receptors [18, 21-23]. Indeed, activation of AMPA/KA receptors induces selective degeneration of motor neurons in vitro [18, 24, 25] and in mice in vivo [26]. This effect is inhibited by selective AMPA/KA receptor antagonists in vitro [21-23, 27] and treatment of SOD1 mice with AMPA/KA receptor antagonists in vivo, significantly extends their lifespan [27-29].

AMPA receptors mediate fast neurotransmission and are composed of 4 different subunits, GluR1-4, which confer different functional characteristics [30]. The absence of the GluR2 subunit renders the AMPA receptor calcium permeable [30]. Expression of GluR2 in motor neurons is regulated by surrounding astrocytes, which therefore play an important role in determining the vulnerability of motor neurons to excitotoxicity [31]. A large number of studies have shown that motor neurons express GluR2-containing AMPA receptors [23, 32-35], although there is some evidence for the co-localisation of GluR2-containing and GluR2-lacking AMPA receptors on the same motor neurons [36]. However, defective GluR2 editing, which would render the receptor calcium permeable, has been found selectively in motor neurons in post-mortem spinal cord tissue from ALS patients [37]. Furthermore, a specific reduction in GluR2 expression was recently identified in motor neurons from presymptomatic SOD1 mice, which may render them more susceptible to excitotoxicity [29, 38].

It is likely that the selective vulnerability of motor neurons to excitotoxicity may be related to a higher density of calcium-permeable AMPA receptors, which will result in an increase in agonist-induced calcium influx into these neurons [23, 24, 27, 39]. Reducing the calcium permeability of AMPA/KA receptors by crossing SOD1 mice with mice over-expressing the GluR2 subunit, significantly delays symptom onset and extends their lifespan [40]. In contrast, acceleration in disease course and a shortening of lifespan is seen in SOD1 mice following manipulations that result in an increase in calcium permeability or the total ablation of the GluR2 subunit [41, 42]. However, there is no motor neuron loss in GluR2 knock-out mice, suggesting that either the absence of the GluR2 subunit alone is not sufficient to induce ALS [43] or that compensatory mechanisms can ameliorate the effects of the lack of GluR2 expression in motor neurons.

The vulnerability of specific populations of motor neurons to glutamate-induced excitotoxicity is further exacerbated by a reduced ability to bind calcium. Thus, specific motor neuron populations that appear to be selectively vulnerable to degeneration in ALS, do not express the calcium binding proteins calbindin-D(28k) and parvalbumin, whereas disease-resistant motor neurons, such as occulomotor neurons and motor neurons in the Onuf’s nucleus [44, 45] express high levels of these calcium binding proteins [46, 47]. Motor neurons with a low expression of calcium binding proteins

<table>
<thead>
<tr>
<th>Manipulation of Endocannabinoid System</th>
<th>Dose and Onset of Treatment</th>
<th>Potential Mechanism of Action</th>
<th>Effects in ALS Models</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIN-55,212-2</td>
<td><em>In vivo:</em> 5mg/kg from symptom onset</td>
<td>Activation of CB1 and CB2 receptors</td>
<td>Delayed disease progression in SOD1 mice 4% increase in lifespan of SOD1 mice</td>
<td>[87]</td>
</tr>
<tr>
<td>AM-1241</td>
<td><em>In vivo:</em> 3mg/kg 0.3mg/kg from symptom onset</td>
<td>Selective CB1 receptor agonist</td>
<td>Delayed disease progression in SOD1 mice Extended lifespan of SOD1 mice by 11%</td>
<td>[84]</td>
</tr>
<tr>
<td>AM-1241</td>
<td><em>In vivo:</em> 1mg/kg presymptomatically</td>
<td>Selective CB2 receptor agonist</td>
<td>Delayed disease progression in SOD1 mice Extended lifespan of SOD1 mice by 7%</td>
<td>[84]</td>
</tr>
<tr>
<td>Δ²-THC</td>
<td><em>In vivo:</em> 20mg/kg from symptom onset</td>
<td>Activation of CB1 and CB2 receptors, also receptor independent anti-oxidative actions</td>
<td>Delayed disease progression in SOD1 mice and extended lifespan by 5%</td>
<td>[85]</td>
</tr>
<tr>
<td>Δ³-THC</td>
<td><em>In vitro:</em> 0.5μM</td>
<td>Activation of CB1 and CB2 receptors, also receptor independent anti-oxidative actions</td>
<td>Neuroprotective against kainate mediated toxicity in motor neurons in vitro</td>
<td>[53]</td>
</tr>
<tr>
<td>Cannabinol</td>
<td><em>In vivo:</em> 5mg/kg presymptomatically</td>
<td>Non-psychoactive cannabinoid. Binds to CB1 and CB2 receptors but with lower affinity than Δ²-THC</td>
<td>Delayed disease onset in SOD1 mice</td>
<td>[145]</td>
</tr>
<tr>
<td>Faah ablation</td>
<td><em>In vivo:</em> From conception</td>
<td>Elevated AEA levels</td>
<td>Delayed disease progression in SOD1 mice</td>
<td>[87]</td>
</tr>
<tr>
<td>CB1 receptor ablation</td>
<td><em>In vivo:</em> From conception</td>
<td>Ablates potential neuroprotective contribution of CB1 receptors</td>
<td>Extended lifespan in SOD1 mice by 13%</td>
<td>[87]</td>
</tr>
</tbody>
</table>

Several manipulations of the endocannabinoid system have been tested in models of ALS, with varying results. This table summarises the results of these studies to date.
may therefore have to depend to a greater extent on mitochondrial calcium uptake in order to buffer excess calcium, and this may render their mitochondria more susceptible to damage, a common feature in ALS. In support of the proposal that a decrease in calcium buffering capacity contributes to the selective vulnerability of motor neurons in ALS, it has been found that over-expression of parvalbumin protects motor neurons in vitro against KA-induced calcium influx [48], and protects neonatal motor neurons from injury-induced motor neuron death in parvalbumin over-expressing transgenic mice [49]. Furthermore, the survival of SOD1 mice crossed with parvalbumin over-expressing transgenic mice is also extended [50].

Together, these findings highlight excitotoxicity as a significant pathogenic mechanism in motor neuron degeneration in ALS and, as discussed above, strategies that reduce the effects of excitotoxicity by antagonising AMPA/KA receptors or by elevating the expression of EAAT-2 (GLT-1) or calcium binding proteins, have beneficial effects in models of ALS. However, despite the fact that cannabinoids have been shown to be neuroprotective in a number of experimental models of excitotoxicity [51-59], until recently, few studies have examined the possible beneficial effects of cannabinoids in ALS. Indeed, due to the activity-dependent nature of their synthesis, it is possible that endocannabinoid synthesis represents an endogenous defence mechanism that is upregulated under excitotoxic conditions [60, 61]. Following injection of excitotoxins into the CNS of mice, levels of the endocannabinoids, N-arachidonoyl ethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) significantly increase [55, 62-64]. This effect may be mediated by activation of postsynaptic group I metabotropic glutamate receptors (mGluR), which will elevate intracellular calcium levels and activate phospholipase C, subsequently inducing endocannabinoid formation (reviewed in [65]). Elevations in endocannabinoids in response to excitotoxicity suggest an adaptive role for endocannabinoids in protection against excitotoxicity. These anti-excitotoxic actions represent a very significant mechanism by which the endocannabinoid system may have therapeutic value in ALS.

Cannabinoid-mediated neuroprotection against excitotoxicity is predominantly the result of activation of CB1 receptors [66] (see Fig. 1) CB1 receptors are coupled to inhibitory GTP binding proteins Goα. Thus their activation can inhibit adenylate cyclase. The resultant reduction in cyclic AMP levels and subsequent inhibition of protein phosphorylation, may inhibit neuronal activity via activation of A-type potassium channels [67]. Furthermore, activation of CB1 receptors mediates inhibition of voltage gated N- and P/Q-type calcium channels [68-71] and activation of inwardly rectifying potassium channels [70], independently of adenylate cyclase inhibition. This presynaptic regulation of calcium and potassium permeability will raise the threshold for neurotransmitter release, thereby decreasing overall release. Indeed in vitro, cannabinoids have been shown to inhibit the release of neurotransmitters [72-74]. In addition, activation of postsynaptic CB1 receptors may also reduce excitotoxicity-induced postsynaptic calcium influx via inhibition of voltage gated N- and P/Q-type calcium channels [53, 68-71, 75]. Therefore under conditions of excitotoxicity, activation of CB1 receptors may reduce both presynaptic glutamate release and the excitability of the postsynaptic cell. Furthermore, the endogenous ligand AEA and the synthetic cannabinoid receptor agonist WIN55,212-2, have been shown to directly interact with and inhibit, sodium channels and T-type calcium channels, actions which would also reduce neuronal excitability and further hinder action potential propagation [74, 76].

Several other signalling pathways may also contribute to the effects of CB1 receptor activation. Activation of the CB1 receptor leads to induction of mRNA expression of immediate early genes, such as c-fos, involved in neuroprotection against excitotoxicity [55], and Krox 24, a growth related gene [77], an effect that is mediated by G protein-dependent activation of extracellular signal regulated kinase [55] and mitogen activated protein kinase respectively [77].

Thus, CB1 receptor activation can potentially inhibit the release and postsynaptic effects of excitotoxic levels of glutamate, therefore providing protection against excitotoxicity. In support of this, it has been shown that injection of kainate (KA) into the central nervous system (CNS) of CB1 receptor knock-out mice produces a more extreme behavioural reaction and a greater mortality than in wild-type (WT) mice [55, 57]. More specifically, Marsicano et al. (2003) generated a conditional knock-out mouse model in which only CB1 receptors on glutamatergic neurons of the forebrain were deleted. Administration of KA to these mice induces seizures that are significantly worse than in mice with a full complement of CB1 receptors. These results imply that CB1 receptors, particularly those located on glutamatergic neurons, play a role in endogenous protection against excitotoxicity and represent a system that may be suitable for therapeutic manipulation by administration of exogenous cannabinoid agonists.

The CB1 receptor is the most predominant cannabinoid receptor subtype in the CNS [78] with the highest densities found in areas including the basal ganglia, the molecular layers of the cerebellum and in portions of the hippocampus [79]. In cultured motor neurons, CB1 receptors are expressed on the cell soma and neurites [53], although the levels of expression in the ventral horn of the spinal cord are relatively low compared to the basal ganglia [79]. Cultured astrocytes and microglial have also been shown to express CB1 receptors [80-82], suggesting that during disease progression, overall CB1 receptor density in the spinal cord may increase due to the influx of microglia to areas affected in ALS (reviewed in [83]). Indeed, CB1 receptor mRNA expression is significantly increased at a symptomatic stage of disease in SOD1 mice, although this is not maintained at disease end-stage [38, 84].

In view of the significant role that excitotoxicity plays in ALS pathogenesis together with the ability of the endocannabinoid system to modulate excitotoxic mechanisms, it has been suggested that cannabinoids may have significant neuroprotective effects in ALS. Certainly, there is now significant evidence to suggest that in models of ALS, cannabinoids can be neuroprotective through anti-excitotoxic mechanisms. Treatment with Δ9-tetrahydrocannabinol (Δ9-THC) protects mouse spinal cord neurons in vitro against direct KA-induced toxicity, an effect blocked by SR141716A, a selective CB1 receptor antagonist [53]. Furthermore, treat-
Cannabinoids may also exert anti-excitotoxic effects in a non-CB$_1$ receptor dependent manner. In the brain of CB$_1$ receptor knock-out mice, an as yet unidentified, G-protein coupled cannabinoid “CB,” receptor has been pharmacologically characterised [89, 90]. This receptor is activated to the same extent by AEA and synthetic cannabinoid WIN55,212-2, but not by other synthetic cannabinoids or Δ$^2$-THC. It is insensitive to CB$_1$ and CB$_2$ receptor antagonists and is not coupled to the inhibition of adenylate cyclase [90], unlike the existing cannabinoid receptors. There is, however, evidence to suggest that this receptor may be selectively coupled to the inhibition of glutamate release in the mouse hippocampus [91]. Further investigation is required to confirm whether activation of this receptor may also have beneficial effects under excitotoxic conditions in models of ALS.

However, despite the established anti-excitotoxic effects of endocannabinoids, there is also evidence that suggests that the endocannabinoid system may be capable of exacerbating excitotoxicity. For example, in hippocampal neurons, endocannabinoids are released in response to depolarisation and activate CB$_1$ receptors on neighbouring astrocytes. In this case, CB$_1$ receptors coupled to G$_{i/o}$ proteins elevate cyclic AMP levels causing an increase in intracellular calcium levels, thereby stimulating the release of glutamate [92, 93]. This glutamate can subsequent activate N-methyl D-aspartate (NMDA) receptors on neighbouring pyramidal neurons [94]. These results show that potentially hyperexcitable motor neurons can release elevated levels of endocannabinoids [86, 87], which under conditions where G$_{i/o}$ protein signal transduction pathways are inhibited, may ultimately contribute to neuronal degeneration, albeit indirectly. This finding may explain the general inability of cannabinoids, which have an element of CB$_1$ receptor activation in their mechanism of action, to extend the lifespan of SOD1 mice despite initially displaying a neuroprotective effect [85, 87].

**TARGETING OF INFLAMMATION BY THE ENDO-CANNABINOID SYSTEM IN ALS**

Over recent years, increasing evidence has highlighted the significant contribution that inflammation plays in ALS pathogenesis. Examination of post-mortal spinal cord tissue from ALS patients shows that there are substantial signs of inflammation including significant proliferation and accumulation of activated microglia, reactive astrocytes and CD4$^+$ and CD8$^+$ lymphocytes in areas of motor neuron degeneration (reviewed in [83]). Furthermore, increases in mRNA and protein levels of inflammatory markers such as COX2 and PGE$_2$ are also seen in post-mortal spinal cord tissue [95, 96]. Similar neuroinflammatory changes are also seen in SOD1 mice and these appear to correlate with disease progression, so that for example reactive astrogliosis increases steadily from symptom onset. However, microglial activation is evident from a presymptomatic age and continues to increase in intensity throughout disease progression, paralleling the loss of motor neurons [97-99]. In fact, increased expression of intracellular adhesion molecule-1 (ICAM-1) and NADPH oxidase, the main reactive oxygen species (ROS) producing enzyme in inflammation, on microglia are some of the earliest pathological changes seen in the SOD1 mice, and this up-regulation may be important in the induction of inflammatory processes [98, 100, 101].

Activated microglia and astrocytes release a variety of neurotoxic mediators including pro-inflammatory cytokines, such as tumour necrosis factor α (TNFα) and interleukin-1β (IL-1β), as well as ROS and glutamate [81, 102, 103]. These neurotoxic agents elicit cellular damage and initiate the recruitment and activation of further glial cells, thus propagating the inflammatory response [81, 104]. Microglia from SOD1 mice have been shown to secrete significantly more TNFα than microglia from age-matched WT mice [99]. This is reflected in an up-regulation of TNFα mRNA and protein in the CNS of SOD1 mice [105]. Pro-inflammatory cytokines can induce COX2 expression, via activation of the transcription factor NF-κB [106]. COX2 activation will then increase production of prostaglandins, including PGE$_2$, which may actually contribute to excitotoxicity by stimulating the release of glutamate from astrocytes [107]. In addition, COX2 activation releases ROS, which may further propagate the inflammatory process [106].

An elevation in COX2 and NF-κB levels has been observed in post-mortem spinal cord tissue from ALS patients in reactive astrocytes [96, 108]. In vitro, in response to cytokine stimulation, SOD1 astrocytes produce substantially more nitric oxide (NO) than WT astrocytes [109]. NO can exert significant neuronal toxicity, via inhibition of mitochondrial respiration, the release of glutamate and subsequent depletion of cellular ATP [110]. Furthermore, NO reacts with superoxide radicals to generate peroxynitrite, which can nitrate tyrosine residues exerting substantial cellular damage. Accordingly, levels of 3-nitrotyrosine are elevated in motor neurons in post-mortem spinal cord tissue from ALS patients [111].

Therapeutic targeting of inflammation has been shown to be particularly effective in models of ALS. Minocycline and nordihydroguaiaretic acid (NDGA), both potent inhibitors of microglial activation and pioglitazone, an agonist of peroxisome proliferator-activated receptor γ, exert anti-inflammatory effects and significantly extend the survival of SOD1 mice [112-116]. Furthermore, selective inhibitors of COX2 delay disease onset [117] and also extend the lifespan of SOD1 mice [118]. Cannabinoids may also exert significant anti-inflammatory effects, mediated primarily by activation of CB$_2$ receptors. Indeed, cannabinoids have been shown to be neuroprotective in several experimental models of inflammation [56, 57, 102, 104, 119]. Interestingly, COX2 acts to degrade endocannabinoids [120], therefore it is possible that the neuroprotective effect of COX2 inhibitors in ALS
may be at least partly attributable to reduced degradation of endocannabinoids [121].

CB₂ receptors were originally regarded as peripheral cannabinoid receptors with high expression levels in the spleen and thymus [122-124]. Recently, however CB₂ receptors have been identified on neurons throughout the CNS, although at significantly lower levels than CB₁ receptors [123, 124]. CB₂ receptors are also expressed by microglia [81, 82, 119]. Interestingly, in response to peripheral nerve injury there is a simultaneous increase in the presence of activated microglia and the expression of CB₂ receptors in the rat spinal cord [125]. CB₂ receptor expression is also upregulated in microglia in post-mortem spinal cord tissue from ALS patients [126].

CB₂ receptors, like CB₁ receptors, are coupled to inhibitory G_i/o proteins. Therefore, activation of CB₂ receptors acts to inhibit cellular activity via stimulation of A-type potassium channels [67]. As illustrated in Fig. (1), cannabinoids acting via a CB₂ receptor dependent mechanism can therefore limit the propagation of the inflammatory response by inhibiting microglial activation [127, 128] and subsequently reducing the expression and release of pro-inflammatory cytokines [81, 104]. Stimulation of microglia in vitro with lipopolysaccharide induces an up-regulation of TNFα and IL-1β mRNA expression in microglia [81, 104]. Treatment with cannabinoid receptor agonists, however, dose-dependently reduces the expression and release of pro-inflammatory cytokines, via a CB₂ receptor-mediated mechanism [119].

In SOD1 mice, treatment with WIN-55,212-2, a cannabinoid agonist which has a slightly higher affinity for CB₂ receptors than CB₁ receptors [129], significantly delayed the onset of motor deficits and motor neuron degeneration [87] and extended lifespan, albeit to a small extent [84] (Table 1). Similarly, ablation of the fatty acid amid hydrolase (Faah) enzyme, which acts to hydrolyse AEA, and subsequent elevation of endocannabinoid levels [130] also delays disease progression in SOD1 mice [87]. However, it is likely that the beneficial effects of both the exogenous cannabinoid WIN-55,212-2 as well as elevated endocannabinoid levels are mediated via the CB₂ receptor. Indeed, genetic ablation of the CB₁ receptor in SOD1 mice has no effect on the disease symptoms and yet significantly extends their lifespan (Table 1). This suggests that the CB₁ receptor may not be involved in the neuroprotective effects mediated by cannabinoids in SOD1 mice and that blockade of the CB₂ receptor may actually have beneficial effects. In support of this, the effects of a selective CB₂ receptor agonist, AM-1241, were recently examined in SOD1 mice. Functional CB₂ receptor expression is up-regulated in the lumbar spinal cord of SOD1 mice from a presymptomatic stage of disease and this up-regulation is maintained with further disease progression [84]. Intraperitoneal administration of the CB₂ receptor agonist AM-1241, which has been shown to be effective in inflammatory models [131], significantly delayed disease onset in SOD1 mice, although this effect was only seen in male mice [132], with a moderate extension in lifespan [84] (Table 1). These results suggest that cannabinoids, acting via the CB₂ receptor, may exert therapeutic effects in ALS models most likely by inhibiting microglial activation and reducing inflammation.

TARGETING OF OXIDATIVE STRESS BY THE ENDOCANNABINOID SYSTEM IN ALS

Several lines of evidence indicate the involvement of oxidative stress in the pathogenesis of ALS. In motor neurons in post-mortem spinal cord tissue from sporadic ALS and familial ALS patients, levels of 3-nitrotyrosine are elevated [111, 133] and in SOD1 mice, free 3-nitrotyrosine immunoreactivity and markers of lipid peroxidation are elevated even at a presymptomatic stage [97, 134]. Furthermore, evidence from SOD1 mice suggests that oxidative damage occurs to both mitochondria [135] as well as EAAT2 (GLT-1) glutamate transporter proteins [136, 137]. In contrast, there is no evidence of protein bound nitrotyrosine in either SOD1 mice or post-mortem tissue from ALS patients [134]. Similarly no increase in hydroxy radical production can be detected in SOD1 mice [134]. Therefore, although there is evidence for oxidative damage in ALS, the mechanism by which it arises remains unknown. In familial ALS, mutations in the SOD1 enzyme structure may increase aberrant interactions with abnormal substrates such as peroxynitrite or hydrogen peroxide, or alternatively impede the binding of copper or zinc ions to the enzyme, although research in this field is conflicting (reviewed in [2]).

It is therefore possible that anti-oxidant agents that reduce oxidative stress may be an effective therapy in ALS. In 1998, Hampson and colleagues reported a potent anti-oxidant capacity of Δ⁹-THC and cannabidiol (CBD), via a cannabinoid receptor-independent mechanism, comparable to the anti-oxidant butylated hydroxytoluene. Furthermore CBD, a non-receptor binding cannabidiol, has greater neuroprotective effects following an excitotoxic insult than vitamin E, an established anti-oxidant [138]. Meanwhile, Δ⁹-THC and CP 55,940 show receptor-independent anti-oxidant activity in vitro, in response to oxidative stress mediated by serum deprivation or hydrogen peroxide exposure, respectively [55, 139]. The therapeutic benefits mediated by this anti-oxidant action of cannabinoids have yet to be established in models of ALS.

CONCLUSIONS

At the current time the only therapy licensed for use in ALS patients is Riluzole, an anti-glutamatergic agent, which unfortunately has only limited therapeutic effects, extending patient lifespan by 2-4 months [140, 141]. In addition to excitotoxicity, several other neurotoxic pathways are implicated in ALS pathogenesis including inflammation and oxidative stress as discussed above. However, ALS is a particularly complex, multi-factorial disorder, which evidence now shows also involves deficits in axonal transport, mitochondrial damage and protein aggregation (reviewed in [2]). This multi-factorial nature of ALS pathogenesis suggests that strategies that target multiple pathways in multiple cell populations, including neurons and glia, may have greater therapeutic benefit than strategies that target individual mechanisms within specific cells types. It is becoming an increasing widely held view that “cocktail” or “combination” therapies will be necessary if a disease modifying therapy for ALS is to be developed. This proposition is supported by results that show that the combination of riluzole, minocycline, an inhibitor of microglial activation, and nimodip-
ine, a blocker of voltage-gated calcium channels, delays disease onset and extends survival in SOD1 mice to a greater extent than achieved individually with these agents [142].

In this regard, agents that are capable of modulating several pathogenic mechanisms may be particularly effective in complex neurodegenerative disorders such as ALS. Cannabinoids have been shown to exert anti-excitotoxic, anti-inflammatory and anti-oxidative effects in several experimental neurodegenerative models (for example, [51-59, 102, 104, 119, 138, 139]). In view of the involvement of these mechanisms in ALS, it is possible that agents that target the endocannabinoid system may be particularly effective in this disorder. However, although agents acting at the CB1 receptor delay disease progression in SOD1 mice, it is only activation of the CB2 receptor, i.e. primarily targeting inflammation, which significantly extends their lifespan (Table I) [84, 132]. This may be related to the fact that although up-regulation of CB1 receptors occurs in symptomatic SOD1 mice, this is not maintained at end-stage, in contrast to the maintained elevation in CB2 receptor levels throughout disease duration [84]. Further investigation is however required to evaluate the full neuroprotective potential of agents that target the CB2 receptor in order to establish whether the use of cannabinoids as a multi-targeted therapy is more effective in ALS than selective targeting of the CB2 receptor. Furthermore, it will be important to establish whether the increase in endocannabinoid levels that occurs during disease progression in ALS represents an adaptive endogenous neuroprotective mechanism. To assist in this, pharmaceutical agents that inhibit endocannabinoid uptake (VDM11; [143]), and AEA hydrolysis (Faah inhibitor, URB597; [144]), are available. In view of the increasing body of evidence that demonstrates the neuroprotective potential of the endocannabinoid system in ALS, a full investigation of the therapeutic potential of these agents in ALS is now justified.

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ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-AG</td>
<td>2-Arachidonoylglycerol</td>
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<td>AEA</td>
<td>Anandamide</td>
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<td>ALS</td>
<td>Amyotrophic Lateral Sclerosis</td>
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<td>AMPA</td>
<td>α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrate</td>
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<td>NDGA</td>
<td>NORDihydroguaiaretic acid</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl D-aspartate</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SOD1</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumour necrosis factor α</td>
</tr>
<tr>
<td>WT</td>
<td>Wild-type</td>
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REFERENCES

References 146-148 are related articles recently published.


The Endocannabinoid System in Amyotrophic Lateral Sclerosis


