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The neuroprotective role of endocannabinoids against chemical-induced injury and other adverse effects

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ABSTRACT: Considerable progress has been made, recently, in understanding the role of the endocannabinoid system in regard to neuroprotection. Endogenous cannabinoids have received increasing attention as potential protective agents in several cases of neuronal injury. The endocannabinoid system is comprised of cannabinoid receptors (CB1 and CB2), their endogenous ligands (endocannabinoids) and proteins responsible for their metabolism. Endocannabinoids serve as retrograde signalling messengers in GABAergic and glutamatergic synapses, as well as modulators of post-synaptic transmission, interacting with other neurotransmitters, including norepinephrine and dopamine. Furthermore, endocannabinoids modulate neuronal, glial and endothelial cell function and exert neuromodulatory, anti-excitotoxic, anti-inflammatory and vasodilatory effects. Physiological stimuli and pathological conditions lead to differential increases in brain endocannabinoids that regulate distinct biological functions. The purpose of this review is to present the available *in vivo* and *in vitro* experimental data, up to date, regarding the endocannabinoid system and its role in neuroprotection, as well as its possible therapeutic perspectives. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: endocannabinoids; 2-AG; AEA; glutamate; neurotoxicity; neuroprotection

Introduction

Cannabinoids, first discovered in the 1940s, are a class of chemical compounds which include the phytocannabinoids (oxygen-containing C_{21} aromatic hydrocarbon compounds found in the cannabis plant) and chemical compounds which mimic the actions of phytocannabinoids or have a similar structure. Synthetic cannabinoids encompass a variety of distinct chemical classes: the classic cannabinoids are structurally related to Δ^9-tetrahydrocannabinol (Δ^9-THC) and the non-classic ones, including the aminoalkylindoles, 1,5-diarylpyrazoles, quinolines and arylsulphonamides, as well as eicosanoids, are related to the endocannabinoids. Δ^9-THC (the primary psychoactive component of the cannabis plant), cannabidiol (CBD) and cannabinol (CBN) are the most prevalent natural cannabinoids and have been studied the most. Δ^9-THC, which has approximately equal affinity for the CB1 and CB2 receptors, appears to ease moderate pain (analgesic) and to be neuroprotective. Cannabinoids can be administered by smoking, vaporizing, oral ingestion, a transdermal patch, intravenous injection, sublingual absorption or rectal suppository. Once in the body, most cannabinoids are metabolized in the liver, especially by cytochrome P450 (CYP) mixed-function oxidases, mainly CYP 2C9 (Grotenhermen, 2005; Pertwee, 2005).

Numerous investigations have revealed the existence of an endogenous lipid signalling system with cannabimimetic actions, refered to as the endocannabinoid system (ES). Recent pharmacological advances have enabled the study of the physiological roles played by the ES and have opened up new strategies in the treatment of various neurological diseases. The purpose of this review is to present current knowledge about the protective role of the ES against neuronal damage of diverse aetiology and consequently the future perspectives of developing potential therapeutic, neuroprotective agents.

The Endocannabinoid System

The ES is involved in a variety of physiological processes including nociception (pain sensation), appetite, lipid metabolism, gastrointestinal motility, cardiovascular modulation, motor activity, mood and memory (Izzo and Sharkey, 2010; Lichtman, 2000; Rodriguez de Fonseca *et al.*, 2005). It is comprised of cannabinoid receptor type-1 (CB1) and type-2 (CB2), which are seven-transmembrane, G-protein coupled receptors, negatively coupled to adenylyl cyclase and positively coupled to extracellular signal-regulated kinase (ERK) [a specific subgroup of mitogen-activated protein kinase (MAPK)] and, especially, p42/p44 (ERK activation pathways) (Howlett and Shim, 2000; Guzman *et al.*, 2001; Matsuda *et al.*, 1990). It also includes their endogenous lipid-based ligands (the endocannabinoids), of which anandamide (N-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) are best defined (Bisogno *et al.*, 2008; Devane *et al.*, 1992; Pertwee and

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Ross, 2002), and the proteins that are responsible for their biosynthesis, transport and degradation (Bari *et al.*, 2006).

CB1 receptors are of the most abundant in the mammalian brain, but they are also expressed in peripheral tissues (Marsicano *et al.*, 2003; Rajesh *et al.*, 2007, 2008). They are highly expressed in brain areas involved in nociceptive transmission and processing including the periaqueductal grey (PAG), anterior cingulate cortex (ACC) and thalamus in addition to the dorsal horn of the spinal cord and dorsal root ganglion (Farquhar-Smith *et al.*, 2000; Herkenham, 1991). CB1 receptors are found on central and peripheral neurons, where they typically mediate the inhibition of amino acid and monoamine neurotransmitter release, such as gamma aminobutyric acid (GABA) (Iversen, 2003; Matyas *et al.*, 2006).

CB2 receptors in the brain are expressed primarily in perivascular microglial cells (Carrier *et al.*, 2004; Gong *et al.*, 2006) and astrocytes (Onaivi *et al.*, 2006; Sheng *et al.*, 2005), where they modulate immune responses (Cabral *et al.*, 2008; Sagredo *et al.*, 2009). They are also expressed in cerebromicrovascular endothelial cells (Golech *et al.*, 2004) and in central (brainstem) and peripheral neurons (Ashton *et al.*, 2006; Van Sickle *et al.*, 2005; Wotherspoon *et al.*, 2005), as well as on the cells of the immune system throughout the whole body (i.e. thymus, spleen, lymph nodes, B-lymphocytes, macrophages and polymorphonuclear cells) (Galiegue *et al.*, 1995; Schatz *et al.*, 1997).

Endocannabinoids are endogenous metabolites of eicosanoid fatty acids. They are lipid signalling mediators of the same CB receptors that mediate the effects of Δ^9-THC (McAllister and Glass, 2002; Mackie, 2006). They are derivatives of arachidonic acid (AA) conjugated with either ethanolamine or glycerol. Apart from AEA and 2-AG, which are the best described, endocannabinoids also include N-arachidonoyl dopamine (NADA), 2-arachidonoylglyceryl ether (2-AGE, noladin ether) and O-arachidonoylethanolamine (OAE, virodhamine) (Devane *et al.*, 1992; Huang *et al.*, 2002; Porter *et al.*, 2002) (Fig. 1).

AEA, the first endocannabinoid to be identified (Devane *et al.*, 1992), appears to be a partial agonist for CB1 receptor (Sugiura *et al.*, 2000) with modest affinity [Ki = 61 nM (rat) and 240 nM (human)] and a relatively weak CB2 receptor ligand (Ki = 440–1930 nM for rodent and human CB2 receptors) with low overall efficacy. AEA is also an agonist for the transient receptor potential vanilloid 1 (TRPV1) (De Petrocellis and Di Marzo, 2005; Di Marzo and Petrosino, 2007; Zygmunt *et al.*, 1999). Recent data suggest that it might also interact directly with other molecular targets, including non-CB1, non-CB2 G-protein coupled receptors (Di Marzo *et al.*, 2000; Sagan *et al.*, 1999), gap junctions (Venance *et al.*, 1995) and various ion channels (Szoke *et al.*, 2000).

2-AG, the second identified CB receptor ligand (Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995), is the most abundant endocannabinoid in the central nervous system (CNS) and a full agonist for both CB1 and CB2 receptors (Di Marzo and Petrosino, 2007; Mackie, 2006; Sugiura *et al.*, 2000) with lower affinity (Ki = 472 and 1400 nM, respectively) and greater efficacy relatively to AEA (Janero *et al.*, 2009; Vemuri *et al.*, 2008).

NADA, discovered in 2000, preferentially binds to the CB1 receptor (Bisogno *et al.*, 2000) and elicits a host of cannabimimetic effects (which include analgesia after systemic administration). Like AEA, NADA is also an agonist for the TRPV1 (Bisogno *et al.*, 2005). It is noteworthy that NADA, through the activation of TRPV1, causes hyperalgesia when administered peripherally (Huang *et al.*, 2002), whereas TRPV1 activation by AEA typically causes analgesia. The distribution pattern of endogenous NADA in various brain areas differs from that of AEA, with the highest levels found in the striatum and hippocampus (Huang *et al.*, 2002). It also exists in the dorsal root ganglion at low levels. Given that NADA is capable of eliciting analgesia upon systemic administration and hyperalgesia upon intradermal injection, it is possible that endogenous NADA may activate either CB1 or TRPV1 depending on location and circumstance.

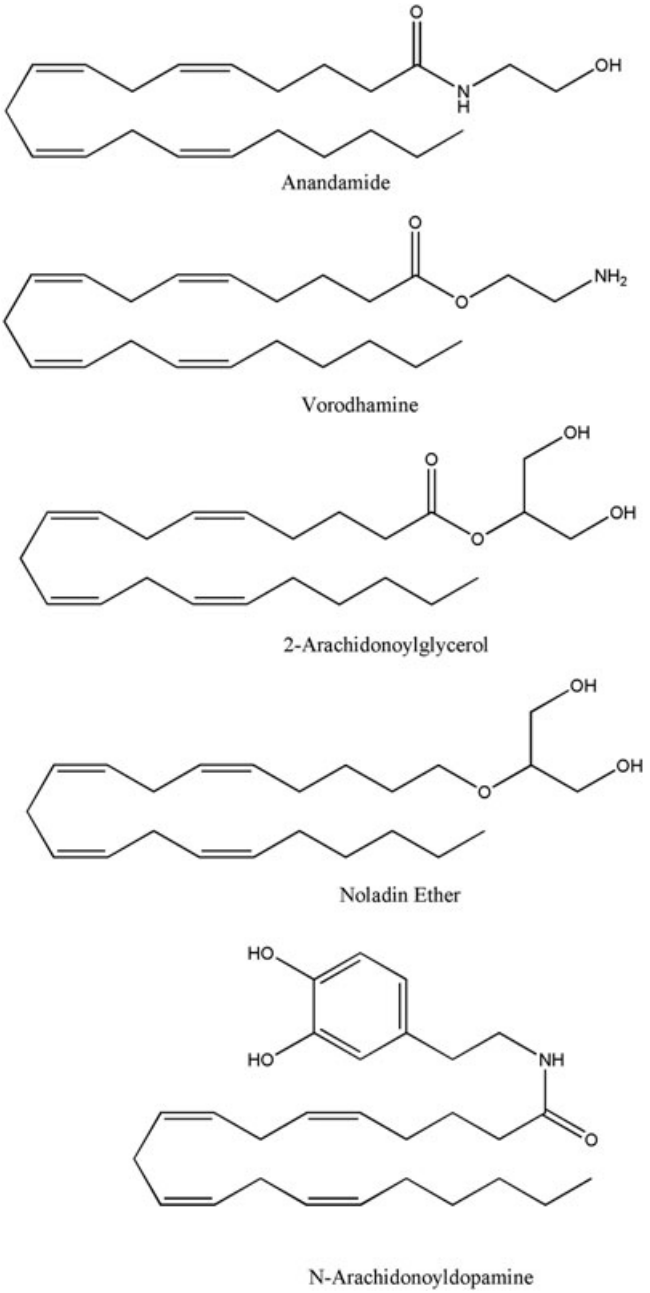

Figure 1. Main endocannabinoids.

2-AGE, isolated in 2001 from porcine brain (Hanus *et al.*, 2001), binds primarily to the CB1 receptor (Ki = 21.2 nmol l^{-1}), and only weakly to the CB2 receptor. It causes sedation, hypothermia, intestinal immobility and mild antinociception in mice (Grotenhermen, 2005).

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OAE, discovered in 2002, is a compound similar to AEA in being formed from AA and ethanolamine, but OAE contains an ester linkage rather than AEA's amide linkage. Although it is a full agonist for the CB2 receptor and a partial agonist for the CB1 receptor, it behaves as a CB1 antagonist *in vivo*. In rats, OAE was found to be present at comparable or slightly lower concentrations than AEA in the brain, but two- to nine-fold higher concentrations peripherally (Porter *et al*., 2002).

Endocannabinoids Biosynthesis and Metabolism

Unlike traditional neurotransmitters, such as acetylcholine and dopamine, endogenous cannabinoids are not stored in vesicles after synthesis, but are synthesized on demand from phospholipid precursors residing in the cell membrane in response to a rise in intracellular calcium levels (Di Marzo *et al*., 1999). However, some evidence suggests that a pool of synthesized endocannabinoids (namely, 2-AG) may exist without the requirement of on-demand synthesis (Longhua *et al*., 2011).

Endocannabinoid levels are elevated in the brain parenchyma as part of internal repair responses to traumatic brain and spinal cord injuries (Garcia-Ovejero *et al*., 2009; van der Stelt *et al*., 2001). Enzymatic synthesis of both AEA and 2-AG draws upon pools of membrane phospholipids such as phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylinositol 4,5-bisphosphate (Ahn *et al*., 2008; Lovinger, 2008). It is worth mentioning that hormones of the gonadal axis (such as estradiol) regulate the expression of the enzymes involved in the synthesis and metabolism of endocannabinoids in different peripheral tissues (López Rodríguez *et al*., 2011).

AEA and its precursor, N-arachidonoylphosphatidyl-ethanolamine (NAPE), are normally expressed at low concentrations in the brains of rats, but increase in a calcium-dependent manner post mortem (Schmid *et al*., 1995) and after severe neuronal injury (Hansen *et al*., 2001; Sugiura *et al*., 2000). A two-step biosynthesis pathway of AEA has been suggested, involving the sequential action of a calcium-dependent transacylase (Ca-TA, N-acyltransferase) that transfers a fatty-acyl chain from a membrane phospholipid molecule onto the primary amine of membrane, phosphatidylethanolamine, to generate NAPE, and a NAPE-selective phospholipase D (NAPE-PLD) that hydrolyzes NAPE to N-acylethanolamines (NAEs) such as AEA (Cadas *et al*., 1997; Natarajan *et al*., 1983, 1984) (Fig. 2). AEA can also be formed by stimulation of dopamine D2 receptors in a G-protein-coupled process (Giuffrida *et al*., 1999).

2-AG is synthesized from diacylglycerol (DAG) by diacylglycerol lipase (DAGL). DAGL, which has been found to modulate after neuronal injury (Wotherspoon *et al*., 2005; Zhang *et al*., 2003), catalyzes the hydrolysis of DAG, releasing a free fatty acid and monoacylglycerol, which is then converted to 2-AG (Piomelli, 2003; Sugiura *et al*., 2004) (Fig. 3). AEA levels increase is followed by 2-AG upregulation (Garcia-Ovejero *et al*., 2009). The accumulation of 2-AG at the site of neuronal injury has been described to be at its peak at 4 h post-injury (Mechoulam *et al*., 2007).

Endocannabinoids serve as retrograde signalling messengers in GABAergic and glutamatergic synapses, as well as modulators of post-synaptic transmission, interacting with other neurotransmitters, including norepinephrine and dopamine (Miller and Walker, 1995). 2-AG and AEA are removed from the extracellular space and transported into cells through a diffusion-facilitated transporter system or uptaken via a membrane-associated carrier and simple diffusion (Croxford and Yamamura, 2005). Thus, endocannabinoid signalling functions are efficiently terminated by cellular uptake and rapid, enzyme-catalyzed hydrolytic inactivation (Di Marzo, 2008; Fegley *et al*., 2004). Fatty acid amide hydrolase (FAAH) (Cravatt *et al*., 1996) and monoacylglycerol lipase (MAGL) (Blankman *et al*., 2007) are the primary catabolic enzymes of AEA and 2-AG, respectively.

FAAH is highly expressed by neurons in the mammalian brain (as an integral membrane protein) and is upregulated after neuronal injury (Wotherspoon *et al*., 2005; Zhang *et al*., 2003). It is localized in the endoplasmic reticulum (ER) of the hippocampus, neocortex and cerebellum (Ahn *et al*., 2008; Egertova *et al*., 1998) and catalyzes the hydrolysis of several endogenous, biologically active lipids, including AEA, oleoyl ethanolamide (OEA) and palmitoyl ethanolamide (PEA)(Karbarz *et al*., 2009). AEA has a short half-life, as it is rapidly hydrolyzed by FAAH and its resting concentrations in the CNS are very low. FAAH degrades AEA into AA and ethanolamine, after its release from neurons (Cravatt *et al*., 2001; Di Marzo, 2008). Enhanced cannabinoid signaling can be achieved by preventing AEA hydrolysis/inactivation by FAAH. A number of FAAH inhibitors exist that can increase the level of AEA in the brain of experimental animals (Ahn *et al*., 2008).

On the other hand, FAAH has been also demonstrated to catalyze AEA synthesis from AA and ethanolamine, with a reported Km for ethanolamine of at least 36 mM (Katayama *et al*., 1999). Several researches have shown that recombinant FAAH protein is capable of catalyzing the reverse of the hydrolase reaction [acting as an AEA synthetase if the concentration of ethanolamine is very high (100 mM)] (Arreaza *et al*., 1997; Kurahashi *et al*., 1997).

2-AG is hydrolyzed into AA and glycerol by either FAAH or, preferably, by monoacyl-glycerol lipase (MAGL) (Di Marzo *et al*., 1999; Karbarz *et al*., 2009; Walter and Stella, 2004). 2-AG has been shown to be a substrate for FAAH both *in vitro* (Cravatt *et al*., 1996; Goparaju *et al*., 1998) and *in vivo* (Maione *et al*., 2007).

Recent evidence reveals that endogenous cannabinoids are also substrates for cyclooxygenase (COX) and can be selectively oxygenated by a COX-2 pathway to form new classes of prostaglandins (prostaglandin glycerol esters and prostaglandin ethanolamides) (Sang and Chen, 2006; Sang *et al*., 2007; Yu *et al*., 1997). Therefore, this is another pathway in degrading endocannabinoids in addition to their well-known hydrolysis pathways. Metabolites of AEA and 2-AG, derived from COX-2, possess biological activity, including the activation of protein kinase C (PKC), as well as having effects on the contractility of smooth muscle preparations (Ross *et al*., 2002; Nirodi *et al*., 2004). Prostanoids derived from both AEA and 2-AG are significantly more stable metabolically than free acid Pgs, suggesting that COX-2 action on endocannabinoids may provide oxygenated lipids with sufficiently long half-lives to act as systemic mediators or pro-drugs (Kozak *et al*., 2004; Patrignani *et al*., 2005).

Endocannabinoids Signalling Pathways and Molecular Targets

Several previous studies have shed light on the mechanism(s) by which cannabinoids produce neuroprotection mediated by CB1 receptors. *In vivo* and *in vitro* data have indicated that the CB1 receptor is involved in the production of neurotrophic factors

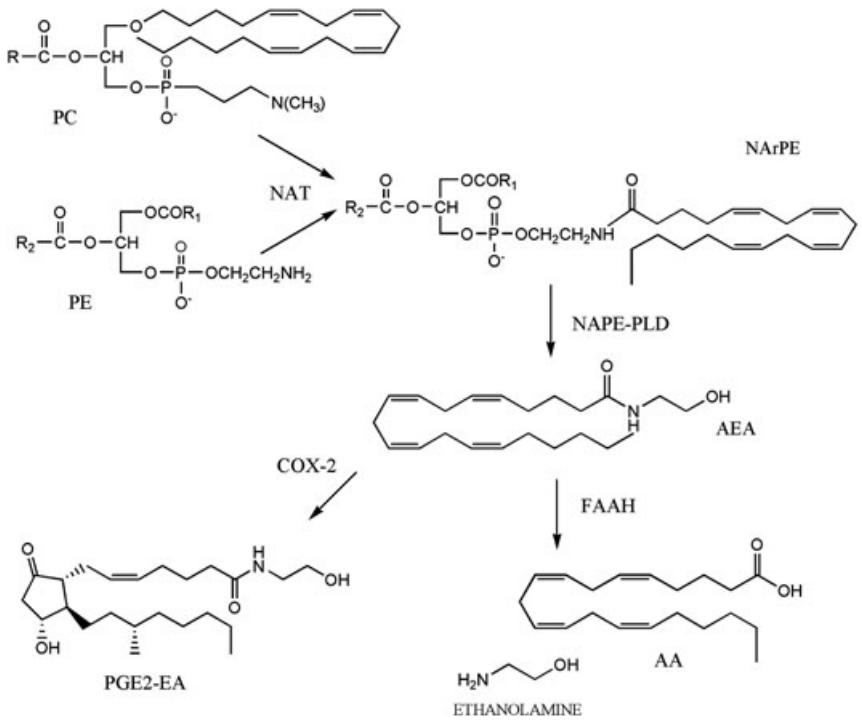

Figure 2. The N-arachidonoylethanolamine (AEA) biosynthesis pathway.

such as basic fibroblast growth factor (bFGF) and brain-derived neurotrophic factor (BDNF) in an excitotoxicity model (Aguado *et al*., 2007; Marsicano *et al*., 2003), the production of nitric oxide (NO) (Kim *et al*., 2006), the inhibition of nuclear factor-kappa B (NF-κB) and of the expression of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) (Panikashvili *et al*., 2005, 2006), and the attenuation of the induction of COX-2 (Zhang and Chen, 2008), all of which may be of importance in determining the outcome of the neurotoxic insult.

Experimental data suggest that the ES contributes to the consequences of cerebral ischaemia via multiple mechanisms. Cannabinoids, as highly lipophilic compounds, can readily penetrate the blood–brain barrier and access the brain (Cabral *et al*., 2008). After that, they induce hypomotility and hypothermia (both of which result in reduced oxygen demand), thus improving hypoxia tolerance and protecting against ischaemia/reperfusion injury (IRI) (Tam *et al*., 2011). Downregulation of certain matrix metalloproteinases (MMPs) may, also, exert neuroprotection. MMP-9 participates in the disruption of the blood–brain barrier during haemorrhagic transformation and exacerbates brain injury after cerebral ischaemia (Mori *et al*., 2002).

Cannabinoids exert their effects through induction of apoptosis, inhibition of cell proliferation, suppression of cytokine production and induction of T-regulatory cells. One major mechanism of immunosupression by cannabinoids is the induction of cell death

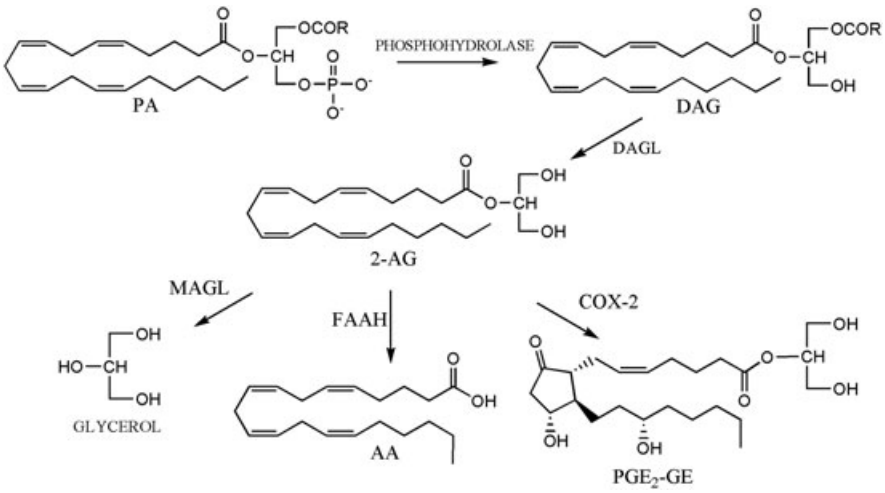

Figure 3. The 2-AG biosynthesis pathway.

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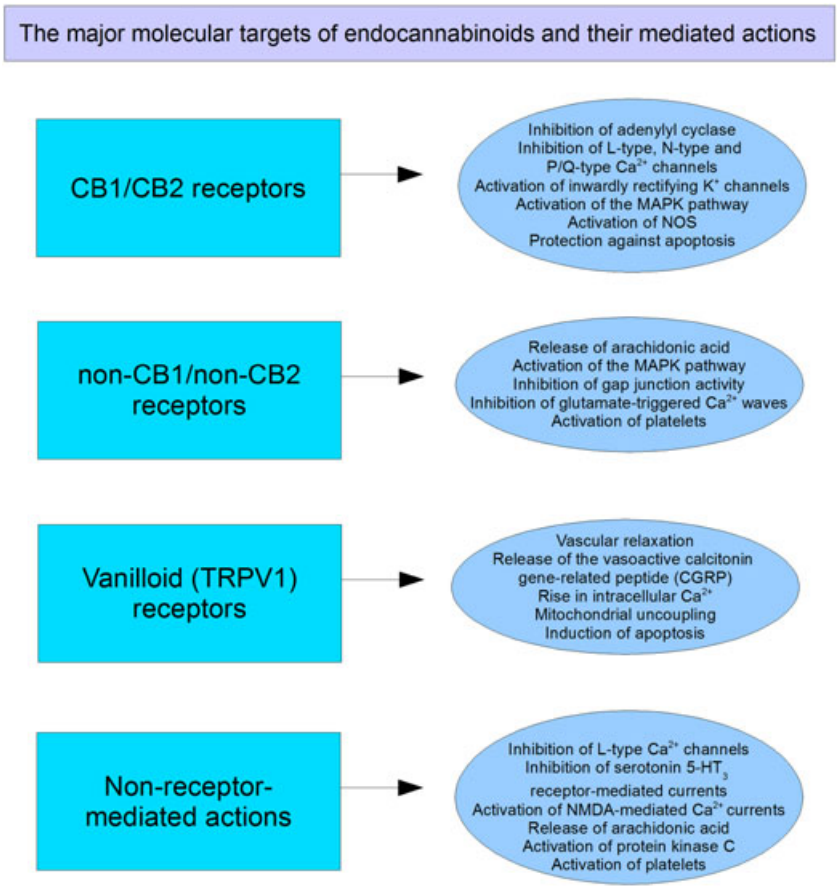

Figure 4. The major molecular targets of endocannabinoids and their mediated actions.

or apoptosis in immune cell populations, thus playing a protective role in autoimmune conditions such as multiple sclerosis (Hengartner, 2000).

In vitro and *in vivo* studies have shown that cannabinoids can act on glia and neurons to inhibit the release of pro-inflammatory cytokines [TNF-α, interleukin (IL)-6 and IL-1β] and enhance the release of anti-inflammatory factors such as the cytokines IL-4 and IL-10 (Facchinetti *et al*., 2003; Sheng *et al*., 2005; Shohami *et al*., 1997a, 1997b). AEA, via the activation of CB1 receptors, enhances the IL-6 synthesis which has both pro- and anti-inflammatory properties, and reduces the synthesis of the pro-inflammatory cytokine TNF-α in Theiler's virus infected astrocytes (Molina-Holgado *et al*., 1998).

CB receptors initiate different signalling pathways including adenylyl cyclase and PKA inhibition and the regulation of ionic channels (Fig. 4). CB1 agonists reduce calcium influx by blocking the activity of voltage-dependent N-, P/Q- and L-type calcium (Ca^{2+}) channels (Choi and Lovinger, 1996; Twitchell *et al*., 1997). This leads to reduced activity of neuronal nitric oxide synthase (nNOS) but also to the reduction of other potentially damaging reactive oxygen species (ROS) (Mehta *et al*., 2007; van der Stelt *et al*., 2002). CB1 activation can also initiate the opening of inwardly rectifying K^+ channels and the inhibition of adenylyl cyclase activity, resulting in a decrease in cytosolic cAMP (Chevaleyre *et al*., 2006; Howlett and Fleming, 1984). In addition, the regulation of neuronal gene expression by CB1 receptors depends on the recruitment of complex networks of intracellular protein kinases, such as the phosphatidylinositol 3-kinase/Akt, the ERK and the focal adhesion kinase (FAK), which become activated, in experimental studies, when hippocampal brain tissue is treated with cannabinoid agonists (Derkinderen *et al*., 1996, 2003). CB1 receptors also modulate the generation of sphingolipid-derived signalling mediators and cell death pathways (e.g. caspase activation and the ER stress response) (Guzman, 2003).

AEA can inhibit a number of different ion channels (Oz, 2006) and it appears that there is a direct extracellular binding site for AEA on these channels. In the brain, the Kv1.5 channel is involved in activation of microglial and dendritic cells and in the proliferation of human glioma cells (Mullen *et al*., 2006; Pannasch *et al*., 2006; Preussat *et al*., 2003). Inhibition of Kv1.5 channels may be immunosuppressive and inhibit glioma cell growth. Kv4.3 channels are found in hippocampal interneurons and in pyramidal and GABAergic cortical neurons where they may be involved in rhythmic activity and controlling synaptic plasticity (Bourdeau *et al*., 2007; Burkhalter *et al*., 2006). Some of AEA's effects are mediated via the CB1 and CB2 receptors, whereas others may involve the action at additional targets such as the TRPV1 ion channel (Karbarz *et al*., 2009). AEA has been demonstrated to activate TRPV1 channels both *in vitro* and *in vivo* and to upregulate genes involved in pro-inflammatory/microglial-related responses (Cernak *et al*., 2004; Maccarrone *et al*., 2000). Activation of TRPV1 leads to an increased influx of Ca^{2+} (Szallasi and Blumberg, 1999), glutamate release (Marinelli *et al*., 2002) and a substantial contribution to neuronal excitotoxicity (apoptosis) (Maccarrone *et al*., 2000; Yue *et al*.,

2004). Therefore, AEA has been found to exert neurotoxic effects in rats via the activation of the TRPV1 receptor (Cernak *et al.*, 2004). In addition, AEA can induce an acute release of NO through endothelial TRPV1 activation, which may be responsible for CB-induced vasorelaxation and hence has beneficial, but also detrimental, effects in models of ischaemia (Poblete *et al.*, 2005).

On the other hand, CB1 receptor activation has been found to inhibit NO release by rat glioblastoma cells exposed to neurotoxic stimuli and decrease amyloid-β-induced reactive astrogliosis (Esposito *et al.*, 2001, 2002, 2007). CB2 receptors mediate anti-inflammatory actions of cannabinoids on astrocytes and microglia. In particular, they decrease the activity of antigen-presenting cells (APC) and down-regulate cytokine (IFN-γ, TNF-α and IL-6) production during inflammatory responses in *in vivo* and *in vitro* studies (Klein and Cabral, 2006; Lombard *et al.*, 2007). The anti-inflammatory effects of cannabinoids on glial cells involve the inhibition of NF-κB-induced transcription of pro-inflammatory chemokines and cytokines. More precisely, endocannabinoids, such as 2-AG, exert neuroprotection after traumatic brain injury through the inhibition of intracellular inflammatory signalling pathways, i.e. they inhibit the production of NF-κB or they inhibit the cAMP/PKA pathway and, thus, they decrease the expression of cAMP-responsive genes (Jacobsson *et al.*, 2000; Panikashvili *et al.*, 2005). Moreover, the CB2 receptor might control immune-cell proliferation by coupling to ERK activation (independent of cAMP), via regulation of mkp-1 gene expression by histone H3 phosphorylation. (Sarker *et al.*, 2000). AEA induces rapid phosphorylation of histone H3 on the mkp-1 gene and also induces mkp-1 expression in microglial cells of inflammatory brain lesions, which suppresses NO release and inflammatory damage in living brain tissue (Eljaschewitsch *et al.*, 2006).

Activation of purinergic P2X7 receptors by ATP has been shown to increase the production of 2-AG from microglia and that these cells, in conjunction with invading brain macrophages, probably constitute the main source of endocannabinoids in inflamed brain, as activated microglia are capable of producing larger amounts of endogenous cannabinoids than neurons (Witting *et al.*, 2006). Microglial cells are under strict control in a healthy brain enviroment. Endocannabinoid signalling strongly suppresses the attack of microglial cells on non-damaged neurons, thus preventing endogenous inflammatory damage as a potential non-desired side effect of continuous immune surveillance and therefore, maintaining a protective and healthy CNS micro-enviroment (Eljaschewitsch *et al.*, 2006). In contrast, after CB receptor inhibition, neuronal damage exacerbates. Thus, activation of CB1 and CB2 receptors suppresses a microglial cell attack on healthy brain tissue and therefore, downregulation of the ES might result in a loss of control and an ongoing attack of microglial cells on neurons (Eljaschewitsch *et al.*, 2006). The release of AEA in injured brain tissue might act as a gatekeeper for signal transduction through the ERK pathway and represent an important negative feedback loop within the CNS immune system needed to reduce the extent of the inflammatory response and to limit neurodegenerative immune reactions after primary brain damage (Eljaschewitsch *et al.*, 2006).

The neuroprotective effects of 2-AG are considered to be CB receptor mediated, as exogenous administration of CB receptor antagonists in mouse models of traumatic brain injury, attenuated these effects (Panikashvili *et al.*, 2005). Moreover, no beneficial effects, neither on neurobehaviour nor on oedema formation, were noted after the treatment of CB1(-/-) mice with 2-AG, in contrast to a significant effect on the wild-type (WT) ones (Panikashvili *et al.*, 2001, 2005). 2-AG suppresses the formation of ROS (McCarron *et al.*, 2003) and TNF-α by murine macrophages *in vitro* after stimulation with lipopolysaccharide (LPS) (Gallily *et al.*, 2000). ROS have been shown to play a role in altering blood–brain barrier permeability and the formation of brain oedema induced by trauma. Antioxidants (e.g. nitroxides) have been reported to protect the blood–brain barrier and 2-AG's antioxidant activity has possible effects on the blood–brain barrier (McCarron *et al.*, 2003). The significant reduction of the blood–brain barrier permeability after treatment with 2-AG may explain its effect on oedema, seen at 24 h, and on functional recovery. These findings also suggest that the mechanism by which 2-AG exerts its effect on the blood–brain barrier may involve inhibition of the early (< 4 h) inflammatory response (Panikashvili *et al.*, 2001, 2005). 2-AG has also been shown to inhibit IL-2 expression in activated thymocytes through inhibition of NF-κB (Herring and Kaminski, 1999; Ouyang *et al.*, 1998) and after traumatic brain injury, it exerts neuroprotection, at least in part, through the same mechanism (inhibition of NF-κB transactivation through CB1 receptors) (Panikashvili *et al.*, 2005). 2-AG has been found to mediate neuroprotection not only via the activation of neuronal CB1 receptors, but also via its action on microglial abnormal cannabidiol (abn-CBD)-sensitive receptors (Kreutz *et al.*, 2009). Other effects of 2-AG include the reduction of endothelin-1 (ET-1)-induced Ca^{2+} mobilization, the rearrangement of the cellular cytoskeleton (actin or vimentin) and the phosphorylation of vasodilatory stimulating phosphoprotein (Chen and Buck, 2000). Thus, taken together, the anti-inflammatory and antioxidant properties of 2-AG may either add or synergize to enhance its activity as a neuroprotective agent (Panikashvili *et al.*, 2001, 2005).

Endocannabinoids mainly induce an inhibitory effect on both GABAergic and glutamatergic neurotransmission and neurotransmitter release, although the results are somewhat variable (Pitler and Alger, 1994; Wilson *et al.*, 2001). In some cases, cannabinoids diminish the effects of GABA, whereas in others they can augment the effects of GABA. The effect of activating a receptor depends on where it is found on the neuron: if CB receptors are presynaptic and inhibit the release of GABA, cannabinoids would diminish GABA effects; the net effect would be stimulation. However, if CB receptors are post-synaptic and on the same cell as GABA receptors, they would probably mimic the effects of GABA; in that case, the net effect would be inhibition (Alsasua del Valle, 2006). Endocannabinoids can do that via the phenomenon of depolarization-induced suppression of inhibition (DSI). DSI refers to endocannabinoid-induced suppression of GABAergic synaptic transmission. In DSI, strong depolarization of a postsynaptic neuron induces a release of a signal that acts on the presynaptic CB1 receptor and transiently inhibits the release of GABA. Such retrograde signalling by endocannabinoid-mediated DSI occurs in the hippocampus but has also been shown outside the hippocampus at interneuron-principal cell synapses (Wilson and Nicoll, 2001; Trettel and Levine, 2003). Thereafter, a similar phenomenon was demonstrated for glutamatergic synaptic transmission and was designated depolarization-induced suppression of excitation (DSE) (Freund *et al.*, 2003; Kreitzer and Regehr, 2001). Most synapses in the CNS use glutamate as an excitatory neurotransmitter. Besides its physiological role in normal synaptic transmission and in mechanisms

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that underlie neuronal plasticity, glutamate is responsible for apoptotic and necrotic neuronal death, a process known as 'excitotoxicity' in a number of acute and chronic neurodegenerative diseases (Choi, 1996; Martin *et al*., 1998). Cannabinoids attenuate glutamate-induced injury by inhibiting glutamate release via presynaptic CB1 receptors coupled to G-proteins and N-type voltage-gated calcium channels (Shen *et al*., 1996). 2-AG, but not AEA, is probably a signalling molecule in mediating CB1-dependent DSI or DSE (Mackie, 2006). Also enzymes that synthesize 2-AG are present in postsynaptic dendritic spines, providing direct evidence that 2-AG is synthesized in post-synaptic sites and acts on pre-synaptic CB1 receptors (Katona *et al*., 2006; Yoshida *et al*., 2006). Thus, endocannabinoids, especially 2-AG, are proposed to serve as retrograde messengers in modulating both GABAergic and glutamatergic synaptic transmission (Alger, 2002; Wilson and Nicoll, 2002).

Implication of Cannabinoids in Neurotoxicity: Research Data

Effects of Exogenous Cannabinoids

Exogenous cannabinoids exhibit neuroprotective actions in cultured neuronal cells exposed to excitotoxic insults (Shen and Thayer, 1998; Zhuang *et al*., 2005) and in cerebral ischaemia (Nagayama *et al*., 1999). Neuroprotective effects of cannabinoids, blocked by CB1 receptor antagonists/inverse agonists such as rimonabant, have also been found in *in vivo* models of neuronal injury, such as trauma (Panikashvili *et al*., 2001) and multiple sclerosis (Baker *et al*., 2000). Moreover, the neuroprotective effects after acute neuronal injury have been described for exogenously administered synthetic cannabinoids, such as HU-211 (or dexanabinol), a synthetic cannabinoid that lacks CB1 and CB2 agonist activity (Shohami *et al*., 1997a, 1997b). HU-211 has neuroprotective effects after optic nerve axotomy (Yoles *et al*., 1996). Bay 38-7271, another synthetic cannabinoid agonist, exerts analgesic and neuroprotective effects after traumatic brain injury in rats (Mauler *et al*., 2003).

The reduction in brain temperature by both Δ^9-THC and synthetic cannabinoids has been proposed as an important possible mechanism underlying the neuroprotective effects of endocannabinoids. CB1 receptors located in the pre-optic anterior hypothalamic nucleus have been suggested to be the primary mediators of CB-induced hypothermia (Rawls *et al*., 2002).

It is worth mentioning that, exogenous cannabinoid administration has also been reported to be neurotoxic *in vivo* (Landfield *et al*., 1988). Δ^9-THC has been found to evoke apoptosis through generation of ROS and activation of the stress-activated kinase, c-Jun N-terminal kinase via the CB1 receptor (Campbell, 2001; Chan *et al*., 1998; Downer *et al*., 2003). Furthermore, several studies have identified a pro-apoptotic role of cannabinoids in transformed neural cells (Jacobsson *et al*., 2000; Maccarrone *et al*., 2000; Sanchez *et al*., 1998; Sarker and Maruyama, 2003). Moreover, there is a significant abuse potential, which has hindered their development as therapeutic agents, as exogenous cannabinoids abuse is an important factor of neurotoxicity. (Gardner, 2005; Gourlay, 2005). Nevertheless, the synthetic cannabinoid abn-CBD represents a promising candidate for the treatment of neuronal injury *in vivo* because it does not bind to CB1 and CB2 receptors and may, thus, produce less undesired side effects (Kreutz *et al*., 2009). Therefore, an alternative approach, which may avoid such side effects, is to manipulate the ES.

Effects of Endocannabinoids

Differences might exist between the effects of on-demand production of endocannabinoids and the administration of CB1 agonists. For instance, on-demand localized activation of the ES has been shown to exert a key role in protection against excitotoxic seizures (Marsicano *et al*., 2003), whereas, systemic treatment with high doses of CB1 agonists, or generalized and congenital enhancement of endocannabinoid levels, showed a paradoxical worsening effect under the same conditions (Clement *et al*., 2003). It has been shown that dual blockade of the endocannabinoid-degrading enzymes MAGL and FAAH by selected organophosphorus nerve agents leads to greater than 10-fold elevations in brain levels of both 2-AG and AEA and to robust CB1-dependent behavioural effects that mirror those observed with CB1 agonists (Nomura *et al*., 2008).

Endocannabinoids, primarily by binding to CB receptors, modulate neuronal, glial and endothelial cell function and exert neuromodulatory, anti-excitotoxic (Baker *et al*., 2001; Marsicano *et al*., 2003), anti-inflammatory (Chang *et al*., 2001; Walter and Stella, 2003) and vasodilatory effects, as endocannabinoids increase the diameter of cerebral arterioles and arteries in a CB1 receptor-dependent fashion, indicating that their main cerebrovascular effect is vasodilatation (Hillard, 2000; Parmentier-Batteur *et al*., 2002). The retrograde signalling of the cannabinoid system can substitute for the GABA system in early development, controlling synaptic transmission and preventing epileptic discharges (Bernard *et al*., 2005). Several previous studies have shown that blocking endocannabinoid signals causes synaptic disruption, increases excitotoxic vulnerability and decreases survival responses (Parmentier-Batteur *et al*., 2002; Karanian *et al*., 2005). Correspondingly, enhancing endocannabinoid signaling leads to improved neuronal survival (Marsicano *et al*., 2003; Wolf *et al*., 2010).

Physiological stimuli and pathological conditions lead to differential increases in brain endocannabinoids that regulate distinct biological functions. Physiological stimuli lead to rapid and transient (seconds to minutes) increases in endocannabinoids that activate neuronal CB1 receptors, modulate ion channels and inhibit neurotransmission (Freund *et al*., 2003), whereas pathological conditions lead to much slower and sustained (hours to days) increases in the endocannabinoid tone that change gene expression, implementing molecular mechanisms that prevent the production and diffusion of harmful mediators (Panikashvili *et al*., 2001; Stella, 2004). There are reports of increased levels of AEA in the cerebrospinal fluid and the blood of stroke patients (Schäbitz *et al*., 2002), whereas, on the other hand, plasma 2-AG levels are not affected (Jean-Gilles *et al*., 2009).

Neurotoxicity Stimuli

Endocannabinoids have been demonstrated to exert neuroprotection against ischaemia, traumatic brain injury and inflammation-induced neuronal damage and also against N-methyl-d-aspartate (NMDA)-, β-amyloid-, kainic acid- and glutamate-induced neurotoxicity (Di Marzo and Matias, 2005; Eljaschewitsch *et al*., 2006; Panikashvili *et al*., 2001) (Tables 1 and 2). Furthermore, endocannabinoids have been shown to exert neuroprotection against chemical-induced neurotoxicity (i.e. organophosphorus insecticides and ethanol) (Nomura *et al*., 2008; Pope *et al*., 2010; Rubio *et al*., 2011). The proposed mechanisms include, among others, blockade of microglial activation (Ramirez *et al*., 2005), an increase in brain-

Table 1. Neuroprotective effects of endocannabinoid activation against cerebral damage

Neurotoxic stimulus	Neuroprotective agent	Dosage of neuroprotective agent	Mechanism of neuroprotection	Results	References
In vitro ischaemia	AEA 2-AG	100 nM 1000 nM	Possible TRPV1 activation CB receptor independent	↑ cell viability	(Sinor *et al.*, 2000)
Cerebral ischaemia	WIN 55212-2 (synthetic CB1 receptor agonist)	0.1-1 mg kg^{-1} (i.p.)	CB1 receptor activation CB1-induced hypothermia ↓ glutamate release	↓ neuronal injury ↓ infarct size ↓susceptibility to NMDA neurotoxicity	(Pellegrini-Giampietro *et al.*, 2009)
Traumatic brain injury	HU-211 (Dexanabinol, NMDA-receptor antagonist, synthetic cannabinoid)	25 mg kg^{-1} (i.p.)	NMDA receptor antagonism	Motor function recovery ↓ blood–brain barrier breakdown ↓ cerebral oedema	(Shohami *et al.*, 1993)
Neuroinflammation	AEA	2–10 μM (o.h.s.c.)	CB2 receptor activation Induction of MKP-1 in microglial cells ↓ inflammatory cytokines (i.e. TNF-α, IL-1β, IL-6) ↑ anti-inflammatory factors (i.e. IL-4, IL-10) ↓ microglial activation ↓ NO release	Neuroprotection ↓ neuroinflammatory responses	(Eljaschewitsch *et al.*, 2006)

i.p., intraperitoneally; o.h.s.c., organotypic hippocampal slice cultures.

Table 2. Neuroprotective effects of endocannabinoid activation against chemical-induced injury

Neurotoxic stimulus	Dosage of neurotoxic agent	Experimental neuroprotective agent	Dosage of neuro protective agent	Mechanism of neuroprotection	Results	References
N-methyl-D-aspartate (NMDA)	50 μM (o.h.s.c.)	2-AG	0.001 μM	CB1 receptor activation Abn-CBD receptor activation ↓ Ca^{2+} influx	Neuroprotection ↓ microglial cells accumulation ↓ degenerating neurons	(Kreutz *et al.*, 2009)
β-amyloid peptide (BAP)	3 μl (10 ng μl^{-1})(i.c.v.) 400 pmol	VDM11 (endocannabinoid cellular uptake selective inhibitor) AA-5-HT (N-arachidonoyl-serotonin, selective FAAH inhibitor)	5 mg kg–1 (i.p) 5 mg kg^{-1} (i.p)	↑ endocannabinoid levels	↓ histological damage ↓ neuronal loss and gliosis	(van der Stelt *et al.*, 2006)
AMPA/kainate	10 μg Ibotenate (i.c.) 15 μg S-bromowillardiine (i.c.)	AEA	10 mg kg^{-1} (i.p.)	CB1 receptor activation	Neuroprotection of the cortical plate and white matter	(Shouman *et al.*, 2006)
Ouabain	0.5 μl (1 mM) (i.c.)	AEA	10 mg kg^{-1}	↓ cellular swelling	↓ neuronal injury	(van der Stelt *et al.*, 2001)
Glutamate	10 mM (o.h.s.c.)	WIN 55212-2 (synthetic CB1 receptor agonist)	30 μM	CB1 receptor activation ↓ Ca^{2+} influx ↓ neuronal nitric oxide synthase	Neuroprotection ↓ glutamate-induced excitotoxicity	(Landucci *et al.*, 2011)
Chlorpyrifos	280 mg kg^{-1} (s.c.)	2-AG	7–9nM	↓ cholinergic neuro transmitter release	↓ cholinergic neuro toxicity	(Pope *et al.*, 2010)
Ethanol withdrawal &NMDA	10 μM (NMDA)	HU210 (synthetic CB1 agonist)	1 μM	↓ presynaptic release of glutamate ↓ Ca^{2+} influx	Neuroprotection ↓ glutamate-induced excitotoxicity	(Rubio *et al.*, 2011)
AEA	20 nmol l^{-1} (i.c.v.)	Capsazepine (TRPV1 receptor antagonist)	35 nmol l^{-1} (i.c.v.)	Calpain activation	↓ cognitive deficits ↓ neuronal loss ↓ vasogenic brain edema	(Cernak *et al.*, 2004)

i.p., intraperitoneally; i.c.v., intracerebroventricularly; i.c., intracerebrally; o.h.s.c., organotypic hippocampal slice cultures; s.c., subcutaneous.

derived neurotrophic factor (Khaspekov *et al.*, 2004), a reduction of calcium influx (Nadler *et al.*, 1993) and antioxidant activity (El-Remessy *et al.*, 2003) (Fig. 5).

Ischaemia

Excitotoxicity and stroke can induce neural progenitor proliferation and differentiation as an attempt of neuroregeneration after damage (Aguado *et al.*, 2007). In the adult brain, the generation of new neurons is restricted to discrete areas including the subventricular and the subgranular zone of the dentate gyrus. CB1 receptors localized on axon presynaptic terminals can modulate the release of GABA (Hajos *et al.*, 2000; Katona *et al.*, 1999) or glutamate (Domenici *et al.*, 2006; Nemeth *et al.*, 2008), and their expression has been demonstrated to be increased in models of cerebral ischemia *in vivo* (Jin *et al.*, 2000; Zhang *et al.*, 2008) and *in vitro* (Fernandez-Lopez *et al.*, 2006). CB1 receptor-deficient mice exhibited impaired hippocampal neural progenitor proliferation and neurogenesis after excitotoxicity. Likewise, CB1 receptor blockade by the selective CB1 antagonist rimonabant (SR141716) administration to wild-type mice effectively blocked excitotoxicity-induced neurogenesis. On the other hand, the ES in macrophages can be activated by oxidized low-density lipoprotein (oxLDL) and it might promote the initiation and progression of atherosclerosis, which is a predisposing factor for stroke. The synthetic cannabinoid Win55,212-2 has been shown to increase the cellular cholesterol accumulation, through the activation of the CB1 receptor (Jiang *et al.*, 2009). Therefore, selectively blocking the CB1 receptor can reduce oxLDL accumulation in macrophages and thus, may offer a new strategy for the treatment of atherosclerosis and the prevention of stroke (Jiang *et al.*, 2009).

Increased CB2 receptor expression is seen in the brain of experimental animals after ischaemia or administration of a dopaminergic neurotoxin (Ashton *et al.*, 2007; Price *et al.*, 2009). Mice lacking CB2 receptors are more sensitive to cerebral insults, and CB2 receptor agonists have neuroprotective effects. The beneficial effects of CB2 receptor agonists have been reported in animal models of focal brain damage, such as middle cerebral artery occlusion and cerebellar lesions (Viscomi *et al.*, 2010; Zhang *et al.*, 2007, 2009).

In experimental studies, submicromolar concentrations of AEA protected cells exposed to hypoxia and glucose deprivation (Sinor *et al.*, 2000). In contrast, higher concentrations of AEA may induce neuronal toxicity *in vitro* and *in vivo* (Cernak *et al.*, 2004; Movsesyan *et al.*, 2004), possibly through enhancing PGE_2 and free radical formation by activated astrocytes and microglial cells, thus leading to oxidative stress (Akundi *et al.*, 2005; Candelario-Jalil *et al.*, 2006).

2-AG has also been shown to protect neurons from insults such as excitotoxicity and ischaemia both *in vitro* and *in vivo* (Melis *et al.*, 2006; van der Stelt *et al.*, 2002). Microglial cells that become activated during pathologies such as excitotoxicity and ischaemia are targeted by 2-AG which modulates their migration and proliferation and also inhibits the production and release of proinflammatory cytokines, including TNF-α and the expression of COX-2 (Facchinetti *et al.*, 2003; Zhang and Chen, 2008). Few studies, however, imply that under certain conditions 2-AG may act as a proinflammatory substance (Kishimoto *et al.*, 2006; Oka *et al.*, 2004, 2006).

Traumatic Brain Injury

Endocannabinoids are produced by neural progenitors upon intracellular calcium increase (Piomelli, 2003), and via CB1 receptor activation they promote hippocampal neural progenitor proliferation (Aguado *et al.*, 2005; Jin *et al.*, 2004) and neurogenesis. CB1 receptor expression increases after injury in various *in vivo* models (Jin *et al.*, 2000; Unzicker *et al.*, 2005), and its activation regulates neural cell survival and proliferation (Guzman, 2003; Mechoulam *et al.*, 2002), migration and axonal growth.

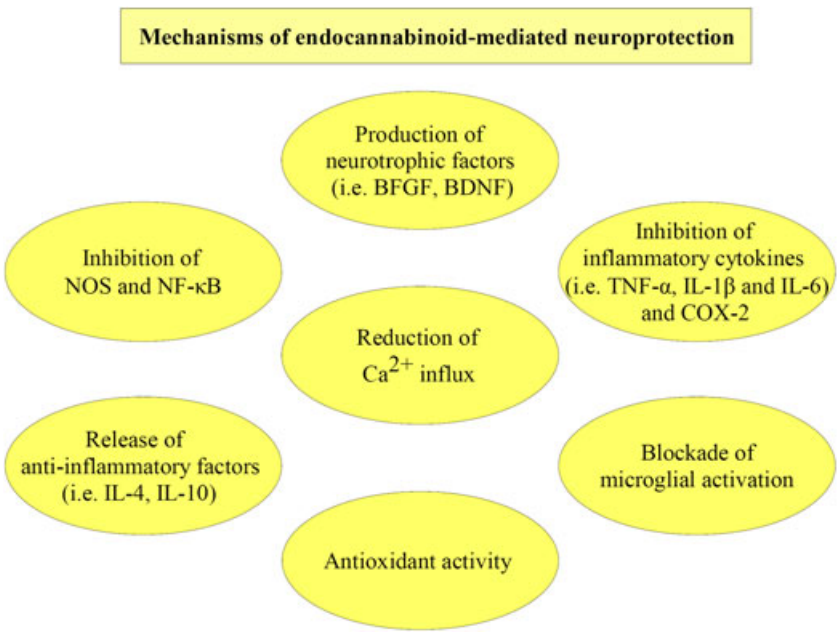

Figure 5. Mechanisms of enocannabinoid-mediated neuroprotection.

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2-AG reduces cerebral oedema and infarct volume, decreases hippocampal cell loss and improves clinical outcome after traumatic brain injury in mice (Panikashvili *et al*., 2001). Furthermore, 2-AG also acts on microglial CB2 receptors and increases their proliferation (Carrier *et al*., 2004). Experiments with CB1 and CB2 receptor-deficient mice have revealed the existence of further, not yet cloned but pharmacologically and functionally well-characterized CB receptors (Mackie and Stella, 2006). The abn-CBD-sensitive receptor is one of these pharmacologically identified non-CB1/non-CB2 receptors and has been first described on endothelial cells of rat mesenteric blood vessels (Wagner *et al*., 1999). This receptor is activated by the endocannabinoid AEA and the synthetic agonist abn-CBD ((2)-4-(3-3,4-trans-p-menthadien-1,8)-yl-olivetol), a derivative of the phytocannabinoid cannabidiol. Abn-CBD-sensitive receptor-mediated effects have also been described for microglial cells: the endocannabinoid 2-AG triggers the migration of microglial cells via activation of the abn-CBD-sensitive receptor (Franklin *et al*., 2003; Walter and Stella, 2003). Moreover, 2-AG attenuates the lipopolysaccharide-induced release of pro-inflammatory cytokines such as TNF-α from microglial cells independently from CB1 and CB2 receptors (Facchinetti *et al*., 2003; Puffenbarger *et al*., 2000).

Neuroinflammation

Neuroinflammation is a biological immune response to various endogenous and exogenous stimuli in the nervous system and localized inflammatory responses in the brain parenchyma have been associated with the pathogenesis and progression of numerous neurological disorders such as infection and ischaemia (Craft *et al*., 2005). At such lesion sites, activated microglia release several types of inflammatory mediators, such as toxic cytokines and ROS that contribute towards the impairment of the blood–brain barrier function and subsequently result in secondary neuronal damage (Liu and Hong, 2003; Walter and Stella, 2004). Among these mediators, prostaglandin E_2 (PGE_2) is of major importance for the initiation, propagation and modulation of brain inflammation. AEA increases PGE_2 and PGD_2 production in activated glial cells (Navarrete *et al*., 2009). Microglia activation and the subsequent release of pro-inflammatory cytokines, ROS and prostaglandins play a role of paramount importance in cerebral damage (Navarrete *et al*., 2009). It is worth mentioning that COX-2 oxidative metabolites of the endocannabinoids may, in some cases, induce neurotoxicity by enhancing excitatory glutamatergic synaptic transmission, thus contributing to the inflammation-induced neurodegeneration (Kozak *et al*., 2004; Sang *et al*., 2007). COX-2-mediated neuronal injury/degeneration is probably attributed to the increased production of AA-derived prostaglandins, mainly PGE_2 (Hurley *et al*., 2002; Kawano *et al*., 2006; Sang *et al*., 2005). While PGE_2 is believed to promote neuronal injury in neuroinflammation, it may also protect neurons from glutamate-induced excitotoxicity and inflammation- or ischaemia-induced neurodegeneration (Akaike *et al*., 1994; Kim *et al*., 2002; McCullough *et al*,. 2004). These contradictory observations suggest that there may be another pathway involved in the COX-2-mediated neurodegenerative process. The PGE_2-G-induced actions are not mediated via a CB1 receptor, but mediated via ERK, inositol 1,4,5-trisphosphate (IP3) and through the phosphorylation of p38 MAPK and NF-κB signal transduction pathways. 2-AG decreases, whereas PGE_2-G increases the frequency of miniature excitatory post-synaptic currents (mEPSCs) (Sang *et al*., 2007). Glutamate receptor antagonists block PGE_2-G-induced neurotoxicity. Inhibition of COX-2 prevents ischaemia or NMDA-induced cell death (Ho *et al*., 1999; Nakayama *et al*., 1998). Elevated neurotoxic PG-Gs and reduced neuroprotective 2-AG are an important mechanism contributing to the COX-2-mediated neurodegeneration during neuroinflammation (Sang *et al*., 2007).

CB2 receptors regulate B- and T-cell differentiation, and the balance of T-helper 1 (Th1) pro-inflammatory to T-helper 2 (Th2) anti-inflammatory cytokines (Ziring *et al*., 2006). In macrophages, CB2 stimulation suppresses proliferation and the release of pro-inflammatory factors such as NO, IL-12 and TNF-α, inhibits phagocytosis, and reduces IL-2 signalling to T-cells (Chuchawankul *et al*., 2004). CB2 activation also suppresses neutrophil migration and differentiation, but induces natural killer cell migration (Nilsson *et al*., 2006).

Nmda-Induced Neurotoxicity

Stimulation of CB1 receptors has been shown to reduce NMDA-receptor-induced excitotoxicity by reducing Ca^{2+} influx and cell death (Shen and Thayer, 1999). Furthermore, microglial activation plays a major role in peri-ventricular white matter lesions induced by agonists acting on NMDA receptors (Tahraoui *et al*., 2001).

β-Amyloid-Induced Neurotoxicity

Interestingly, endocannabinoids, as well as the non-psychotropic cannabinoid, CBD, have been shown to reduce cell toxicity induced by β-amyloid peptide (BAP) fragments (Esposito *et al*., 2005; Iuvone *et al*., 2004). VDM-11, an inhibitor of endocannabinoid cellular reuptake, administered in rodents, 3 days after BAP treatment, entirely reversed the histological damage and the biochemical markers of neuronal loss and gliosis induced by the peptide, as well as the increase in CB2 receptor protein. On the other hand, when the inhibitor was administered 7 days post-BAP treatment, no significant amelioration of the histological and biochemical changes induced by BAP was observed even in the presence of enhanced AEA levels. Therefore, both early and strong pharmacological elevation of brain endocannabinoid concentrations can provide protection against BAP-induced neuronal damage or memory loss in rodents. In contrast, when it is exerted at a later phase of BAP-induced neurotoxicity (or when it is not strong enough), the boosting of brain endocannabinoid levels has no effect on neuronal damage and worsens memory loss in BAP-treated rats and mice, respectively (van der Stelt *et al*., 2006).

Kainic Acid-Induced Neurotoxicity

In a mouse model, endocannabinoids protected the developing white matter and cortical plate in a dose-dependent and long-lasting manner against an AMPA/kainate receptor-mediated challenge. Endocannabinoid-induced neuroprotection of white matter involved increased survival of preoligodendrocytes and increased preservation of myelination (Shouman *et al*., 2006).

Ouabain-Induced Neurotoxicity

Regarding ouabain-induced neurotoxicity which has also been studied, endogenous AEA may only be released after an intense stimulus of ouabain, and, hence, too late to exert a protective action, whereas exogenous AEA may inhibit the ouabain-induced glutamatergic transmission, thereby preventing spreading and reducing the effect of the toxic stimulus (van der Stelt *et al.*, 2001).

Glutamate-Induced Neurotoxicity

CB1 receptors control the excitability and excitotoxicity of glutamate (Marsicano *et al.*, 2003; Monory *et al.*, 2006) and CB1 receptor-deficient mice exhibit increased mortality and a larger infarct size after permanent focal ischaemia (Parmentier-Batteur *et al.*, 2002). The hippocampal slice cultures are widely used to model various neuropathologies owing to their expression of similar signaling, genetic and cellular responses to pathogenic insults as found *in vivo* (Bonde *et al.*, 2005; Jourdi *et al.*, 2009; Vornov *et al.*, 1994). The ES has been found to influence seizure activity in the hippocampus (Monory *et al.*, 2006). It has been suggested in different animal models of epilepsy that high concentrations of CB1 receptors in the hippocampal formation reduce seizure activity by protecting neurons against excessive glutamatergic activity (Araujo *et al.*, 2010; Arida *et al.*, 2005).

Both COX-2 and the enzymes synthesizing 2-AG are present in post-synaptic dendritic spines of excitatory neurons. The colocalization of COX-2 and 2-AG in the same subcellular space allows COX-2 to rapidly and efficiently metabolize 2-AG when COX-2 expression or activity is elevated. Thus, the inhibition of COX-2 prevents the inactivation of endocannabinoids, raising the endocannabinoids levels and promoting the endocannabinoid-mediated response (thus, enhancing neuroprotection), whereas the elevation of COX-2 accelerates the metabolism of endocannabinoids, lowering their levels and attenuating the endocannabinoid-mediated response (Katona *et al.*, 2006; Yoshida *et al.*, 2006). Thus, the elevation of COX-2 activity enhances excitatory glutamatergic neurotransmission (Sang *et al.*, 2005; Yang *et al.*, 2007).

Chemical-Induced Neurotoxicity

Various exogenous, synthetic neurotoxicants, such as organophosphorus insecticides, that primarily act by altering synaptic neurotransmitter levels (inhibition of acetylcholinesterase and elevation of synaptic acetylcholine levels), may be particularly sensitive to the neuromodulatory actions of endocannabinoids. After *in vivo* exposure, the organophosphorus insecticide chlorpyrifos (O,O′-diethyl-3,5,6-trichloropyridinyl-phosphorothioate) more effectively activates endocannabinoid signalling to decrease cholinergic and/or non-cholinergic neurotransmitter release and block the expression of cholinergic toxicity (Pope *et al.*, 2010).

The stimulation of the ES is, also, protective against the hyperexcitability developed during alcohol withdrawal (cessation of chronic ethanol consumption can increase the sensitivity of the brain to excitotoxic damages). In an *in vitro* model of chronic ethanol exposure, ethanol withdrawal increased NMDA-induced neuronal death (Rubio *et al.*, 2011). The stimulation of the ES with the CB agonist HU-210 decreased NMDA-induced neuronal death exclusively in ethanol-withdrawn neurons. This neuroprotection could be explained by a decrease in NMDA-stimulated Ca^{2+} influx after the administration of HU-210. By contrast, the inhibition of the ES with the CB1 receptor antagonist rimonabant (SR141716) during ethanol withdrawal increased death of ethanol-withdrawn neurons without any modification of NMDA-stimulated Ca^{2+} influx (Rubio *et al.*, 2011).

AEA-Induced Neurotoxicity

In vitro studies have demonstrated that both Δ^9-THC and AEA can be toxic to neurons in primary culture, but in concentrations considerably higher than those activating CB receptors (Chan *et al.*, 1998; Movsesyan *et al.*, 2004). AEA has been shown to induce apoptotic cell death in human neuroblastoma CHP100, as also in lymphoma U937 and PC-12 cells (Maccarrone *et al.*, 2000; Sarker *et al.*, 2000). Furthermore, intracerebroventricular administration of AEA in rats causes sustained cerebral, as reflected by diffusion-weighted magnetic resonance imaging, regional cell loss (loss of neurons in the hippocampus measured 24 h later) and an impairment in long-term cognitive function (Cernak *et al.*, 2004). The formation of apoptotic bodies induced by AEA corresponds to increases in intracellular calcium, mitochondrial uncoupling, and cytochrome *c* release (Maccarrone *et al.*, 2000). Central administration of AEA, also, significantly upregulates genes involved in proinflammatory/microglial-related responses. These effects are mediated, in part, through TRPV1 (Maccarrone *et al.*, 2000) as well as through calpain-dependent mechanisms. Nevertheless, several previous studies have revealed that activation of CB1 receptors can also induce cytotoxic effects in a number of cultured cell systems (Downer *et al.*, 2003) including the hippocampal (Chan *et al.*, 1998) and cortical neurons (Downer *et al.*, 2001). Furthermore, the CB1 receptor antagonist rimonabant has also been reported to have neuroprotective properties in a number of animal models of neurodegenerative disorders, thus, implying that the modulation of the ES could contribute towards neuroprotection or neurotoxicity depending on a number of factors (different degrees to which AEA and 2-AG are mobilized, the type of receptor activated and the degree to which related lipids such as PEA are involved) (Fowler *et al.*, 2010). Among suggested mechanisms of cannabinoid-induced neurotoxicity are activation of caspase-3-dependent apoptosis (Campbell, 2001; Downer *et al.*, 2001), generation of ROS (Chan *et al.*, 1998), sustained ceramide accumulation (Galve-Roperh *et al.*, 2002), activation of the JNK cascade (Sarker and Maruyama, 2003) and sphingomyelin hydrolysis (Sanchez *et al.*, 1998). Thus, AEA (as well as Δ^9-THC) can produce neurotoxic effects both *in vitro* and *in vivo* through multiple CB1-receptor-mediated (Downer *et al.*, 2001, 2003) and CB1-receptor-independent mechanisms (Cernak *et al.*, 2004), and whether the final effect of AEA would be neuroprotection or neurotoxicity might be depending on the balance of its action on CB1 receptors on the one hand, and TRPV1 receptors or calcium-mediated signal transduction pathways on the other.

Conclusions

Considerable progress has been made, recently, in understanding the role of endocannabinoids in preventing or reducing the effects of various neurotoxic insults. The ES represents a local messenger between the nervous and immune system and is obviously involved in the control of immune activation and neuroprotection. Manipulation of endocannabinoids and/or the use of exogenous cannabinoids *in vivo* can constitute a potent treatment modality against inflammatory disorders. Cannabinoids

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have been tested in several experimental models of autoimmune disorders such as multiple sclerosis, rheumatoid arthritis, colitis and hepatitis, and have been shown to protect the host from the pathogenesis through induction of multiple anti-inflammatory pathways.

Furthermore, the ES has been shown to mediate neuroprotection in many neurological and psychiatric disorders including pain, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's chorea, multiple sclerosis, amyotrophic lateral sclerosis and epilepsy (Centonze *et al*., 2007a, 2007b; Galve-Roperh *et al*., 2008; Maccarrone, 2008; Pacher *et al*., 2006). It also has neurotrophic and neuroprotective effects in cerebral ischaemia (stroke) and traumatic brain injury (Mechoulam and Shohami, 2007).

Endocannabinoids and exogenously administered CB1 receptor agonists produce beneficial effects in models of *in vitro* (Shen and Thayer, 1998) and *in vivo* ischaemia (Nagayama *et al*., 1999). As most strokes are ischaemic in nature, manipulation of the ES and/or administration of exogenous cannabinoids could be a promising therapeutic option for treating strokes in the future.

Endocannabinoid signalling may be enhanced indirectly to therapeutic levels through FAAH inhibition (thus, prolonging the duration of action of endogenously released AEA), making FAAH an attractive pharmacotherapeutic target and selective FAAH inhibitors attractive drug candidates for various neurological and neurodegenerative/neuroinflammatory disorders (including seizures of diverse aetiology, multiple sclerosis, Alzheimer's, Huntington's and Parkinson's diseases (Benito *et al*., 2003; Bisogno and Di Marzo, 2008; Maccarrone *et al*., 2003; Micale *et al*., 2007; Ramirez *et al*., 2005). The site- and event-specific character of the pharmacological inhibition of endocannabinoid deactivating enzymes such as FAAH and MAGL may offer increased selectivity with less risk of the undesirable side effects that have been observed with CB-receptor agonists capable of activating all accessible receptors indiscriminately (Janero *et al*., 2009; Vemuri *et al*., 2008).

The ES is an emerging target for drug discovery, because it is involved in the regulation of many cellular and physiological functions. The modulation of the ES by selective agonists or antagonists may hold tremendous therapeutic potential in various cases of neurotoxicity. Numerous researches have revealed several secrets of the ES and although, further information is still required before the ES is completely comprehended, its pharmacological modulation seems, nowadays, a viable target which will pave the way for the therapeutic intervention at a wide spectrum of diseases.

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