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REVIEW

Endocannabinoids and traumatic brain injury

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Traumatic brain injury (TBI) represents the leading cause of death in young individuals. It triggers the accumulation of harmful mediators, leading to secondary damage, yet protective mechanisms are also set in motion. The endocannabinoid (eCB) system consists of ligands, such as anandamide and 2-arachidonoyl-glycerol (2-AG), receptors (e.g. CB1, CB2), transporters and enzymes, which are responsible for the 'on-demand' synthesis and degradation of these lipid mediators. There is a large body of evidence showing that eCB are markedly increased in response to pathogenic events. This fact, as well as numerous studies on experimental models of brain toxicity, neuroinflammation and trauma supports the notion that the eCB are part of the brain's compensatory or repair mechanisms. These are mediated via CB receptors signalling pathways that are linked to neuronal survival and repair. The levels of 2-AG, the most highly abundant eCB, are significantly elevated after TBI and when administered to TBI mice, 2-AG decreases brain oedema, inflammation and infarct volume and improves clinical recovery. The role of CB1 in mediating these effects was demonstrated using selective antagonists or CB1 knockout mice. CB2 were shown in other models of brain insults to reduce white blood cell rolling and adhesion, to reduce infarct size and to improve motor function. This review is focused on the role the eCB system plays as a self-neuroprotective mechanism and its potential as a basis for the development of novel therapeutic modality for the treatment of CNS pathologies with special emphasis on TBI.

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Abbreviations*

2-AG, 2-arachidonoyl-glycerol; AraS, *N*-arachidonoyl-L-serine; CB1, *ENSG00000118432*; CB2, *ENSG00000162562*; DGL, diacylglycerol lipase; eCB: endocannabinoid; TRPV1, *ENSG00000043316*; FAAH, fatty acid amide hydrolase; GPR55, *ENSG00000135898*; TBI, traumatic brain injury

Introduction

Over the last two decades, since the endocannabinoid (eCB) system was discovered, our knowledge of its structure and functions has significantly expanded. This system consists of ligands, such as anandamide and 2-arachidonoyl-glycerol (2-AG), receptors (CB1, CB2, possibly also TRPV1 and GPR55), transporters and enzymes, which are responsible for the synthesis [N-acyl-phosphatidylethanolamine-phospholipase D, diacylglycerol lipase (DGL)] and degradation of these lipid mediators [fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase] (Piomelli, 2003; Mackie, 2006).

There is a multiplicity of eCB actions, mainly in the brain, under both physiological and pathological conditions.

Unlike 'classical' neurotransmitters, the eCBs are not stored in presynaptic vesicles, rather, they are produced 'on demand' when increased intracellular Ca^{++} is the major intracellular trigger for synthesis. The primary ligands produced in the brain are anandamide (Devane *et al.*, 1992) and 2-AG (Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995), which activate both the CB1 and CB2 receptors. Brain tissue concentrations of 2-AG are approximately 200-fold higher than those of anandamide (Bisogno *et al.*, 1999) and the rank order for the distribution of both eCB in different areas is similar: highest in brainstem, striatum and hippocampus and lower in cortex, diencephalon and cerebellum. There is a large body of evidence showing that eCB are markedly increased in response to pathogenic events such as kainic acid-induced seizures, 6-hydroxydopamine or glutamate toxicity, shock-induced

stress and trauma. The fact that the eCB system is activated in response to such events suggests that it is part of the brain's compensatory repair mechanism, mediated via CB receptors signalling (for review: Bahr *et al.*, 2006).

The CB receptors belong to the large superfamily of G protein-coupled receptors (GPCR) (Piomelli, 2003; for a recent review see Basavarajappa, 2007), whereas TRPV1 is a ligand-operated cation channel, which is related to the transient potential receptor family of unselective ion channels (Caterina *et al.*, 1997). The neuronal CB1 is the more abundant CB receptor in the brain and is responsible for the psychoactive effects of the cannabinoids. These receptors have been shown to be localized presynaptically on GABAergic interneurons and glutamatergic neurons (Hajos *et al.*, 2000; Hajos *et al.*, 2001; Katona *et al.*, 2001). Further studies have indeed corroborated the role of the eCB compounds in the control of excessive neuronal activity in the brain and in the modulation of neurotransmission (Marsicano *et al.*, 2003) via retrograde signalling associated with inhibition of neurotransmitter release (for review: Onaivi, 2009).

CB2 are expressed predominantly in non-neuronal cells as well as on subpopulations of neurons, yet, they exert no psychoactivity. Although considered to be located mostly in the immune system CB2R are now well recognized on resident inflammatory cells within the CNS, on microglial and dendritic cells (Maresz *et al.*, 2005; Pertwee, 2008) and on brain endothelial cells (Golech *et al.*, 2004). Activation of these receptors attenuates the inflammatory response by inhibiting the release of pro-inflammatory mediators and by diminishing leukocyte chemotaxis and extravasation into the brain parenchyma (Pacher and Hasko, 2008). CB2 agonists were also found to decrease cytocrome-C release, inhibit apoptosis and to exert anti-inflammatory effects in a diverse range of animal models (Ashton and Glass, 2007; Benito *et al.*, 2008).

Vanilloid type 1 (TRPV1) receptors are found not only on sensory neurons, where they are partly co-expressed with CB1 receptors (Ahluwalia *et al.*, 2000) but also in several central nuclei including hypothalamus, basal ganglia, hippocampus and cerebellum (Mezey *et al.*, 2000; Di Marzo *et al.*, 2001). They are also co-expressed with CB1 and CB2 receptors on the cerebromicrovascular endothelial cells, which represent the main component of the blood–brain barrier and are involved in eCB-mediated vasodilation (Golech *et al.*, 2004).

Summing up, there is ample evidence suggesting that the eCBs interact with at least three types of receptors at binding sites located at a variety of cell types in the brain. The specific dominant interaction depends on a number of factors, including the levels of eCBs, tissue receptor distribution and accessibility to the receptors.

Is the eCB system a potential 'self-neuroprotective' entity

The expression and function of the eCBs and their respective receptors in the brain, on neurons, astrocytes, microglia and the cerebrovasculature point to their role in multiple (patho) physiological functions. To explore the role of anandamide signalling *in vivo*, several investigators have targeted its degrading enzyme in order to augment and extend its brain activities. Thus, the role of anandamide in setting an endogenous cannabinoid tone was shown in mice lacking the

enzyme FAAH2/2. Upon administration of exogenous anandamide, its brain levels were augmented 15-fold and the mice exhibited robust, CB1-dependent behavioural responses such as hypomotility, analgesia, catalepsy and hypothermia (Cravatt *et al.*, 2001). These findings attest to the role of FAAH as a key regulator of anandamide signalling *in vivo*. Anandamide also modulates emotional states as described by Kathuria *et al.* (2003). A class of potent, selective and systemically active inhibitors of FAAH, which augment brain levels of anandamide, exhibit benzodiazepine-like properties in stressful situations. These effects are prevented by CB1 receptor blockade, pointing to the eCB as potential mediators of novel anti-anxiety therapy. In a recent review Hwang *et al.* (2010) provide data supporting the notion that selective FAAH inhibitors have therapeutic potential against neuropathological states including traumatic brain injury (TBI) and stroke as well as neurodegenrerative diseases such as Alzheimer's, Huntington's and Parkinson's diseases. The accumulating data demonstrate that neuronal injury activates eCB signalling as an intrinsic neuroprotective response via activating signalling pathways downstream from CB receptors and promote neuronal maintenance and function.

Ischemic and traumatic brain injuries are CNS pathologies in which high intracellular calcium accumulation are among the earliest events. They share a secondary complex of harmful pathways that include excitotoxicity, oxidative stress and acute inflammatory response (Leker and Shohami, 2002). It is now well accepted that the complex response to ischaemia and trauma need to be targeted by drug(s) that can modulate a number of independent injury factors simultaneously (Vink and Nimmo, 2009). The formation and accumulation of eCBs in response to injury, along with their multipotent properties as anti-oxidants, vasodilators, anti-inflammatory agents, inhibitors of excitotoxicity, as well as their role in neurogenesis, suggest that the formation of eCBs may represent a 'self-neuroprotective' and neuroregenerative response. As ischaemia and TBI induce the release of numerous mediators, many of which are harmful [e.g. glutamate, reactive oxygen species (ROS), pro-inflammatory cytokines, etc.], the eCBs stand out as neuroprotectants. Thus, to distinguish them from the endogenous harmful mediators, on one hand, and from exogenous neuroprotective synthetic drugs, on the other, the term 'self-neuroprotective' response describes their 'on-demand' synthesis and accumulation after injury. Thus, the lesson learned from the endogenous, multifactorial neuroprotecitve role of the eCBs can set the ground for the development of novel compounds targeting either receptors, enzymes or transporters involved in the eCB system.

This review is focused on the role the eCB system plays as a self-neuroprotective mechanism and its potential as a basis for the development of novel therapeutic modality for the treatment of CNS pathologies such as TBI and stroke.

eCBs as neuromodulators of excitotoxicity

Over the last two decades, hyperactivation of the NMDA receptors by extracellular excitatory amino acids, such as glutamate, has been implicated in the cellular events leading to neuronal death and decline in function following traumatic or ischemic brain injury (e.g. Hayes *et al.*, 1988; Faden *et al.*, 1989). Acute increases in extracellular glutamate,

detected in both experimental brain trauma models and human patients, may lead to over-stimulation of glutamate receptors, culminating in neuronal damage (Lynch and Dawson, 1994; Alessandri et al., 1996; Brown et al., 1998). Agents modulating glutamate transmission were developed targeting, as antagonists, the NMDA receptors (Tolias and Bullock, 2004; Beauchamp et al., 2008; Kalia et al., 2008), that could theoretically ameliorate the harmful effects of excessive glutamate. Based on the location of the CB1 receptors on presynaptic terminals of glutamatergic synapses, and the inhibitory nature of their signalling, one may suggest a role for eCBs and other CB1 agonists as neuromodulators of glutamate release, hence, as modulators of excitotoxicity following numerous brain disorders. Supporting this notion, Coomber et al. (2008) recently reported that inhibition of eCB metabolism attenuates enhanced hippocampal neuronal activity induced by Kainic Acid. Cannabinoid receptor agonists were shown to inhibit glutamatergic synaptic transmission in rat hippocampal cultures and to protect rat hippocampal neurons from excitotoxicity (Shen et al., 1996; Shen and Thayer, 1998). Anandamide protected cerebral rat cortical neurons from in vitro ischaemia (Sinor et al., 2000) and a synthetic cannabinoid agonist, WIN-55 212 protected rat brain against ischaemia (Nagayama et al., 1999). It was suggested that the protection of neurons against secondary excitotoxicity was due to the closing of calcium channels. Gilbert et al. used rat hippocampal neurons in culture to investigate the effect of CB1 agonists on the survival of neurons exposed to an excitotoxic pattern of synaptic activity (Gilbert et al., 2007). They showed a significant reduction in cell death when cultures were exposed to either tetrahydro-cannabinol (THC) or the synthetic agonist WIN 55212-2. In excitotoxically lesioned organotypic hippocampal slice cultures, the CB1 receptor antagonist AM251 blocked the neuroprotection mediated by WIN (Koch et al., 2010). The transport inhibitor N-(4-hydroxyphenyl)-rachidonamide (AM404) and the FAAH inhibitor palmitylsulphonyl fluoride (AM374) enhanced mitogen-activated protein kinase activation in cultured hippocampal slices. After an excitotoxic insult to the slices, these combined inhibitors protected against cytoskeletal damage and synaptic decline, similar to the effect produced by CB1 agonist Karanian et al. (2005).

However, the CB1-mediated neuroprotection showed desensitization, probably due to receptor down-regulation, after prolonged exposure to the agonists (24 h).

A crucial component of cell survival, activated by CB1 receptors, is the PI3K/Akt pathway. Acute administration of THC increases the Ser473 phosphorylation of Akt in mouse hippocampus, striatum, and cerebellum. This effect is blocked by the selective CB1 antagonist rimonabant (Ozaita et al., 2007). Activation of this pathway could modulate the expression and activity of genes involved in cell survival, highlighting the CB1-induced neuroprotection afforded by endogenous and synthetic CB1 agonists. The synthetic cannabinoid agonist HU-210 was shown to be coupled to extracellular signal; regulated kinase (ERK) activation. It stimulated the PI3K downstream targets protein kinase B (PKB), as shown by its phosphorylation in Thr 308 and Ser 473 residues, and Raf-1 (Galve-Roperh et al., 2002). The findings that CB1-induced ERK activation is mediated by PI3KIB is of important consequences in the control of cell death/survival decision.

Taken together, the activities of 2-AG reported in the literature, prompted us to expect that this eCB might be beneficial in the setting of TBI. Presynaptic Ca^{2+} accumulation, through activated NMDA receptor channels, is one of the early post-injury events, which leads to the activation of phospholipase C, production of diacylglycerol and subsequently of 2-AG (Piomelli et al., 1998). Excess quantities of glutamate in the extracellular space lead to uncontrolled shifts of sodium, potassium and calcium, disrupting ionic homeostasis and leading to severe cell swelling and death. 2-AG, by activating at presynaptic CB1 receptors on nerve terminals, may modify neurotransmission and specifically, inhibit glutamate release, thus limiting its excitotoxicity (Schweitzer, 2000; Schlicker and Kathmann, 2001).

eCB in neuroinflammation

In parallel to, or immediately following, the massive glutamate release after traumatic or ischemic brain injury, there is robust production of ROS, within minutes of injury (Chan, 2001) and the inflammatory cytokines initiating the brain inflammatory response are up-regulated within hours (Shohami et al., 1999). In LPS-stimulated macrophages, a widely used model for an in vitro inflammatory response, 2-AG suppressed the formation of tumour necrosis factor-α (TNF-α) and ROS. Also, using LPS-stimulated mice as in vivo assay, TNF-α was significantly inhibited by 2-AG (Gallily et al., 2000). TNF receptors recruit, upon ligand activation, multiple intracellular adapter proteins that activate the transcription factor NF-κB, a key regulator of the inflammatory response (Karin and Ben-Neriah, 2000). This factor is composed of homo- and heterodimers including p65 which contains a translocation domain in its C-terminal end and p50. Inactive NF-κB is retained in the cytosol where its activity is tightly regulated by members of the IκB family. NF-κB thus released translocates into the nucleus and activates various pro-inflammatory genes. Among its other neuroprotective properties (see below) 2-AG was shown to inhibit NF-κB activation after TBI in a CB1-dependent manner (Panikashvili et al., 2005). Peroxisome proliferator-activated receptors (PPARs) are nuclear membrane-associated transcription factors belong to the nuclear receptor family which exerts anti-inflammatory properties in brain injury. Activation of PPARα suppresses NF-κB, thus creating a negative feedback loop for controlling acute post-traumatic inflammation. A role as neuroprotective/anti-inflammatory agents was recently attributed to cannabinoids by their agonistic action to PPARs (for review: Stahel et al., 2008).

Among the numerous processes in which the eCB system is reported to modulate the inflammatory response via activation of CB2, those relevant to traumatic or ischemic brain injury are leukocyte activation and extravasation into the brain parenchyma. These include rolling, adhesion to the endothelium and transmigration. Indeed, activation of CB2 receptors by synthetic specific agonists (such as O-3853, O-1966) significantly attenuated these processes and afforded neuroprotection in models of ischemic stroke (Zhang et al., 2007; Pacher and Hasko, 2008).

In the brain, CB2 receptors are expressed predominantly in non-neuronal cells, and are up-regulated mainly under neuroinflammatory conditions. Their levels in the brain may also increase under conditions that lead to peripheral

immune cells infiltration. Whereas in health normal expression of CB2 is hardly detected, they are up-regulated in activated microglia (for review: Stella, 2010) leading to increased cell proliferation along with reduction of the release of proinflammatory agents such as TNF-α and NO.

2-Arachidonoyl-glycerol has been shown to increase rat microglial cells proliferation *in vitro* (Carrier *et al.*, 2004) while they also seem to produce 2-AG. Several authors have highlighted the importance of 2-AG on the regulation of microglia during disease (e.g. Franklin and Stella, 2003; Carrier *et al.*, 2004; Benito *et al.*, 2005; Cabral and Marciano-Cabral, 2005; Ehrhart *et al.*, 2005). In view on these data, 2-AG could be considered a potent modulator of CNS diseases where inflammation and autoimmunity are the cause of CNS damage (Centonze *et al.*, 2007).

The nature of the CB receptors, which activate the agonist-mediated response in glia cells is still not fully elucidated, and CB-like receptors are implicated in the regulation of their response. Several reports described the presence of CB-like receptors in cultured astrocyes; however, their role *in vivo* is yet to be determined (Stella, 2010).

eCBs as vasomodulators of the cerebrovasculature

2-AG and the cerebromicrovasculature. 2-Arachidonoyl-glycerol was shown to cause hypotension, which may be attributed to its hyperpolarizing properties (Mechoulam *et al.*, 1998; Varga *et al.*, 1998). It has been suggested that induction of 2-AG release in endothelium occurs in parallel to nitric oxide (NO) and involves activation of cholinergic receptors (Randall and Kendall, 1998). Nitric oxide, the most effective endothelium-derived relaxing factor (EDRF), and endothelial-derived hyperpolarizing factor (EDHF) (Cohen and Vanhoutte, 1995) have a close functional relationship with ET-1 in regulating the endothelial-dependent capillary and microvascular responses in the brain (Chen *et al.*, 1999). In addition, accumulated experimental data indicate that factors other than NO or prostanoids may contribute to EDRF/EDHF-mediated endothelium-dependent relaxation (Cohen and Vanhoutte, 1995). Our study on the effect of eCBs on Ca^{2+} influx in human brain endothelial cells (HBEC) revealed that they stimulate Ca^{2+} accumulation within the cells and alter the cytoskeleton (actin and vimentin) rearrangement (Chen *et al.*, 2000). These responses were partly inhibited by the CB1 receptor antagonist SR141716A, whereas 2-AG-induced vasodilator-stimulated phosphoprotein phosphorylation was predominantly mediated by the TRPV1 receptor, but not by CB1 or CB2 receptors (Golech *et al.*, 2004). 2-AG was also shown to inhibit the ET-1-induced Ca^{2+} influx into HBEC, providing direct evidence of a functional interaction between 2-AG and ET-1 and suggesting a potential alternative pathway for abrogating ET-1-inducible, endothelium-dependent capillary and/or microvascular effects in the brain.

In view of these observations, we have demonstrated that HBEC express CB1, CB2 and TRPV1 receptors, and that 2-AG functions as a vasorelaxant, that may counteract the powerful vasoconstrictor ET-1 (Chen *et al.*, 2000; Golech *et al.*, 2004). Together, these studies provide a pathway for regulating endothelial-dependent vascular reactivity, which may be of importance in pathological conditions, such as TBI, a condi-

tion that leads to release of both vasoconstrictor ET-1 and vasorelaxant eCBs, and specifically 2-AG.

Taken together, the colocalization and functional capacities of TRPV1, CB1 and CB2 receptors on HBEC strongly suggest that these receptors may affect the function of cerebral microvascular endothelium and contribute to the regulation of cerebral blood flow and blood–brain barrier permeability. As these are impaired in stroke and TBI, it appears that the cerebral microvasculature is also targeted and can be protected by the eCB system.

eCBs in neurogenesis. eCBs are detected in rodents from the gestational period, with levels of 2-AG being 1000-fold higher than those of anandamide. Interestingly, while anandamide displayed a gradual increase, 2-AG displayed constant levels throughout development with a single peak on the first postnatal day (Berrendero *et al.*, 1999). At different embryonic and post-natal stages of brain development the eCB system is involved in the regulation of neural progenitors (NP) differentiation, which occurs in parallel with CB1 receptor expression (Aguado *et al.*, 2005; Aguado *et al.*, 2006). CB2 is present in progenitor cells from embryo origin and from adult brain, so it is assumed that CB2 mediate acceleration of neurogenesis and stimulate neural progenitor proliferation (Palazuelos *et al.*, 2006, 2008; Galve-Roperh *et al.*, 2007).

In a recent study, Gao *et al.* (2010) addressed the role of 2-AG in neurogenesis and plasticity using genetic manipulations. They produced mice lacking either DGLα or DGLβ and determined 2-AG levels, hippocampal synaptic plasticity and the degree of neurogenesis in WT and DGL knockout mice. 2-AG levels in DGL$\alpha^{-/-}$ and DGL$\beta^{-/-}$ were 80% and 50% (respectively) lower than in the WT controls, and only DGL$\alpha^{-/-}$ mice completely lost synaptic plasticity. However, both knockout mice had compromised neurogenesis. These findings corroborate the role of DGL in 2-AG synthesis, and the role of 2-AG in synaptic plasticity and neurogenesis.

These findings, along with the expression of CB receptors in adult hippocampus and in neural precursor cells (NPCs) at the subventricular zone point to the possibility of targeting these cells in various pathological conditions in which neural stem cell manipulations may promote recovery, such as ischaemia, TBI and other CNS pathologies which are associated with neuronal cell loss.

eCBs as neuroprotectants in TBI – are they a 'magic bullet'?

Traumatic brain injury is the leading cause of death in the young age group and the most commonly identified cause of epilepsy in adult populations older than 35 years (Finfer and Cohen, 2001). It triggers a cascade of events characterized by the activation of molecular and cellular responses, mostly harmful, leading to secondary injury (Leker and Shohami, 2002). The evolution of the secondary injury that occurs in the area surrounding the site of trauma is an active process in which many biochemical pathways have been identified. Studies of the temporal changes of mediators in the evolution of secondary damage reveal impairment of brain ionic homeostasis and massive glutamate release from presynaptic terminals within minutes to hours after injury (Leker and Shohami, 2002). ROS are massively produced after TBI (Beit-

Yannai *et al.*, 1997; Chan, 2001) and inflammatory cytokines originating in brain resident cells (neurons and astrocytes) accumulate in the brain parenchyma (Shohami *et al.*, 1999). In addition, endothelium-derived active mediators are also released (Barone *et al.*, 2000) affecting the local vascular tone. After brain trauma the net effect of the vasoactive molecules implies an early (~1 h) ischemic episode followed by a late (>24 h) hyperaemia (Assaf *et al.*, 1999).

The observation of these multifactorial events along with the pharmacological profile of the eCBs described above, led over the last decade to investigations, by us and others, of the neuroprotectant role of the eCB system after TBI.

2-AG affords neuroprotection in a mouse model of closed head injury

To address the question on the role of 2-AG in the brain following TBI we designed a study to investigate: a) the dynamic changes in brain 2-AG levels after TBI; b) the possibility that exogenous 2-AG may attenuate brain damage after injury and c) the involvement of the CB1 receptor in neuroprotection (Panikashvili *et al.*, 2001).

Using a mouse model of closed head injury (Shohami *et al.*, 1997) we found that 2-AG levels were already significantly elevated in the ipsilateral hemisphere 1 h after injury, peaking to tenfold increase at 4 h and declining thereafter. Even after 24 h the levels of 2-AG were still higher (600%) than in controls. Treatment with synthetic 2-AG resulted in attenuated oedema formation, infarct volume and blood–brain barrier permeability. In addition, neuronal cells at the CA3 hippocampal region were protected and the neurobehavioural status of the mice at 24 h after injury displayed greater recovery. The CB1 antagonist SR141716A partly inhibited 2-AG protection, albeit at a relatively high dose, suggesting that these effects are not solely mediated via the CB1 receptor (Panikashvili *et al.*, 2001, 2005, 2006).

Pro-inflammatory cytokines play a crucial role in TBI and are released from brain resident cells early (within hours) after injury (Shohami *et al.*, 1997; Ziebell and Morganti-Kossmann, 2010). They are also released from the infiltrating inflammatory cells, invading the brain via the compromised BBB. As 2-AG was shown to inhibit the production and release of TNF-α and IL-6 from LPS-stimulated macrophages (Gallily *et al.*, 2000), we examined its effect in the early post-traumatic period. Indeed, the acute expression of the main proinflammatory cytokines: TNF-α, IL-1β and IL-6 was inhibited in mice treated with 2-AG 1 h after TBI (Panikashvili *et al.*, 2006). We have also demonstrated that inhibition of NF-κB, which occurs within hours after TBI, is associated with significant improved outcome (Beni *et al.*, 2004). We therefore investigated the effect of 2-AG on NF-κB activation after injury and found that treatment with 2-AG completely abolished the robust activation of this transcription factor. Interestingly, the extent of activation of NF-κB in the CB1$^{-/-}$ mice was similar to that observed in the WT, suggesting that the endogenous 2-AG does not affect the inflammatory signalling. However, when treated with exogenous 2-AG, CB1$^{-/-}$ mice did not respond at all to 2-AG treatment, which abolished activation in the WT (Panikashvili *et al.*, 2005). Importantly, the aforementioned effects of 2-AG on the pathophysiology of TBI were accompanied by significant and long-lasting beneficial effect on the functional outcome.

Whereas in our earlier reports the neurological severity score, which is a measure of the functional status of the animal, was evaluated for 2–3 days, we have recently shown that single administration of 2-AG (5 mg·kg^{-1}) 1 h after closed head injury significantly facilitated the recovery of function, an effect that became even more pronounced 3 weeks later. Neurobehaviour function continued to improve until 6 weeks, when maximal recovery was achieved. The level of function that was achieved by 6 weeks sustained until the end of a long-term follow-up, at 3 months (Cohen-Yeshurun *et al.*, 2007).

Originally we focused on the role of CB1 in mediating CB-induced neuroprotection, mainly because there was no evidence to the existence or role of CB2 in the brain.

However, recently, the presence of CB2 was noted in microglia (see above) and brain neuronal and glial processes involving CB2 were investigated (for reviews: Onaivi, 2009; Zhang *et al.*, 2009). Hence the relevance of CB2 in the context of TBI drew our attention.

A novel camphor-based cannabinoid was synthesized in our laboratories. This compound, HU-910, is a selective agonist for CB2 with Ki = 6.0 nM, which is 228-fold higher than that for CB1 and was found to inhibit LPS-induced TNF-α production in macrophages. We therefore treated mice 1 h after infliction of TBI with this drug and their functional status was evaluated for 3 weeks. HU-910-treated mice displayed a trend towards better recovery as compared with vehicle-treated controls already 1 day after injury; by day 3 this difference reached significance, which sustained until the end of the observation period, 3 weeks later (Magid *et al.*, 2009). This effect was abolished by co-administration of a selective CB2 antagonist, SR144528, corroborating the role of CB2 in mediating the protective effect. The anti-inflammatory effect of HU-910 was also evident in the post-TBI brain as the early (2 h) increase in TNF-α production was abolished by the drug in the hippocampus. Interestingly, 25 days after injury, mice treated with HU-910 displayed significant higher levels of synpatophysin in cortical extracts, suggesting a role for CB2 activation in synaptogenesis (Elgali *et al.*, 2009). Further studies on the potential of CB2 agonists in the treatment of TBI are warranted, as they are even more promising than the CB1 agonists for translation to humans because they lack the CB1-mediated psychotropic effects.

Diacylglycerol lipase α and DGLβ are the enzymes responsible for production and maintenance of 2-AG in the brain as well as in other tissues. To address the role of these enzymes in eCB signalling Gao *et al.*, (2010) recently generated gene knockout mice to these specific enzymes and found that 2-AG levels are reduced by up to 80% in the brain and spinal cord of the DGL$\alpha^{-/-}$ and by up to 50% in the brain in the DGL$\beta^{-/-}$ mice. In a preliminary study we examined the levels of both DGL isoforms in the cortex and hippocampus of mice after TBI (Elgali *et al.*, 2009). A transient reduction in the levels of DGLα and DGLβ was found in the hippocampus 1 h after injury, which reverted to normal from 8 h on (until 7 days of follow-up). In the cortex, no changes were found in the levels of DGLβ whereas DGLα levels at 5 h post trauma were significantly elevated, as compared with sham controls. To the best of our knowledge, this is the first, yet preliminary, report on changes in the eCB synthetic enzymes in the setting of
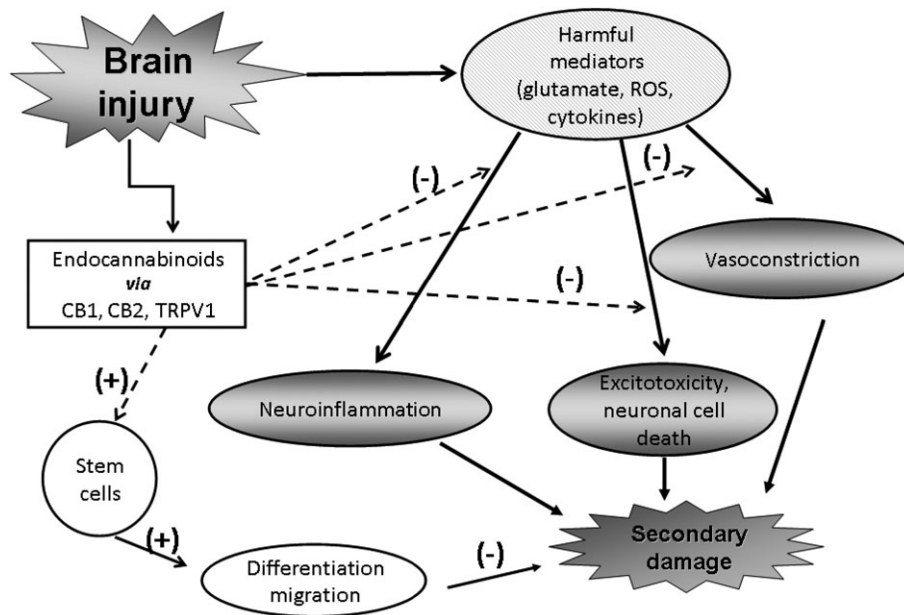
Figure 1

A schematic representation of post-TBI events: pahtophysiological pathways and the role of eCB as neuroprotectants. TBI leads to the release and accumulation in the brain of harmful mediators which act upon all cell types: neurons, astrocytes and brain endothelial cells. These activities lead, respectively, to neuronal cell death, augmented inflammatory response and vasoconstriction, culminating in secondary damage. At the same time, there is 'on demand' synthesis by all brain cell types to produce eCB, which in turn inhibit excitotoxicity, act as antioxidants, inhibit production of the inflammatory cytokines and counteract the vasoconstrictor effects of ET-1. In addition, eCB augment stem cells differentiation and migration. The result of the eCB activity is to attenuate the secondary damage hence they act as 'endogenous neuroprotectants'. Inhibition is designated by (–); enhanced activity is designated by (+). eCB, endocannabinoid; ROS, reactive oxygen species; TBI, traumatic brain injury.

brain injury, and further studies are needed to explore this avenue.

N-arachidonoyl-L-serine – a novel eCB

N-arachidonoyl-L-serine (AraS), is an eCB-like compound, with anandamide-like structure, that was isolated by Milman *et al*. (2006) from bovine brain. AraS produces endothelium-dependent arterial vasodilatation and stimulates phosphory-lation of p44/42 mitogen-activated protein (MAP) kinase and PKB/Akt in cultured endothelial cells (Milman *et al*., 2006). These effects are similar to the stimulation observed following treatment with the classical eCBs (Chen *et al*., 2000; Golech *et al*., 2004) despite the very low binding affinity of AraS to CB1 and CB2 (Milman *et al*., 2006). To date, no known CB receptor has been found to bind this compound and specific binding assays indicated no binding of AraS to GPR55, GPR12 and GPR35 cannabinoid receptor (Cohen-Yeshurun *et al*. submitted for publication). AraS is, however, a low efficacy agonist to GPR18 (McHugh *et al*., 2010). As it is structurally related to the eCB family, we investigated whether AraS, like other eCBs, provides neuroprotection following TBI and examined some of the mechanisms involved. Indeed, a single injection of AraS given 1 h after TBI led to a significant improvement in functional outcome, which sustained during 3-month follow-up, and was comparable with that achieved by 2-AG. It also led to a 40% decrease in lesion volume, compared with vehicle (Cohen-Yeshurun *et al*., 2007, 2009). Specific antagonists to CB2 and to TRPV1 or to the large conductance calcium-activated potassium (BK)

channels but not to CB1 reversed these protective effects. Intracellular signalling was affected by AraS such that pAkt levels were significantly higher than in vehicle controls 4 h after injury, while those of pERK remained high 2 h after injury, in contrast with the decrease in the vehicle controls. Moreover, attenuation of apoptotic processes were also noted at 24 h (higher levels of Bcl-xL) and 3 days (lower caspase 3 activity). These findings agree with those of Milman *et al*. (2006), who showed in several *in vitro* settings that AraS produces endothelium-dependent vasodilation, stimulates phophorylation of Akt and suppresses LPS-induced TNF production. Many of these effects parallel those reported for abnormal, synthetic cannabinoid agonist (abn-CBD), which is an agonist to GPR18 (McHugh *et al*., 2010).

To examine the effect of AraS on proliferation and migration of multipotential NPCs, cortical neurospheres, consisting of NPCs were isolated from E15 mouse brains and grown for evaluation of proliferation and migration for 4 and 5 days respectively. When different doses of AraS were added to the cultures medium, the size of the neurospheres was increased in a dose-dependent manner. The AraS-treated neurospheres also showed a significant migratory effect compared with vehicle-treated cultures (Cohen-Yeshurun *et al*., 2009). Collectively, our data on AraS *in vivo* and *in vitro* support the notion that this novel eCB family member exerts neuroprotective, as well as neuroregenerative properties, and further studies into its role after TBI are warranted.

In conclusion, we have demonstrated that the eCB system, by acting at each of the components of the neurovas-

cular unit, has the ability to affect the functional outcome after TBI by a variety of mechanisms. The scheme shown in Figure 1 depicts the interrelationship between the harmful mediators that lead to secondary brain damages after TBI, and the neuroprotecitve effects exerted by the eCB within hours of injury. These involve inhibition of excitatory neural transmission, inhibition of the inflammatory response and reducing vascular tone. The delayed, neuroregenerative effects are probably mediated via NPC proliferation. The synthetic cannabinoids which mimic the activities of anandamide, 2-AG, Ara-S and other family members, as well as synthetic specific CB2 agonists, should be considered candidates for the development of novel drugs for the treatment of TBI.

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Conflict of interest

There is no conflict of interests.

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