Cannabinoids and brain injury: therapeutic implications

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Mounting *in vitro* and *in vivo* data suggest that the endocannabinoids anandamide and 2-arachidonoyl glycerol, as well as some plant and synthetic cannabinoids, have neuroprotective effects following brain injury. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission and reduce the production of tumour necrosis factor-α and reactive oxygen intermediates, which are factors in causing neuronal damage. The formation of the endocannabinoids anandamide and 2-arachidonoyl glycerol is strongly enhanced after brain injury, and there is evidence that these compounds reduce the secondary damage incurred. Some plant and synthetic cannabinoids, which do not bind to the cannabinoid receptors, have also been shown to be neuroprotective, possibly through their direct effect on the excitatory glutamate system and/or as antioxidants.

Traumatic brain injury (TBI) is a leading cause of death in young people, particularly in males. No specific therapy is available, because of the lack of understanding of the pathological mechanisms that are involved in the development of secondary damage. Numerous treatments – corticosteroids, mannitol, barbiturates, hyperventilation, cerebrospinal fluid drainage and hypothermia – are employed today to reduce the neurological damage caused by TBI. However, a critical evaluation of the literature on the first five treatments mentioned [1] and clinical experimental work on hypothermia [2] have shown that none of these methods brings significant improvement. There are preliminary indications that progesterone could be of value in post-injury treatment of TBI [3]. Obviously, novel approaches are urgently needed and, indeed the clinical importance of TBI has led to a large number of investigations on the mechanisms of the secondary damage produced by the injury, as well as on the endogenous neuroprotective, restorative mechanisms available to the injured brain.

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Excitotoxicity, produced primarily by high concentrations of glutamate, and activation of glutamate receptors, is widely accepted as a central process in secondary damage and cell death. This is mainly due to the intracellular accumulation of cytotoxic levels of calcium, which leads to activation of numerous destructive pathways, with reactive oxygen intermediates (ROI), calpains and caspases taking part in various processes [4]. A putative mediator that might contribute to focal ischemia following TBI is endothelin (ET). ET is now well known to play a significant role in the cerebral circulation. It produces vasoconstriction to reduce blood flow *via* ET-A receptors, and it contributes to the pathophysiology of ischemic and hemorrhagic stroke [5]. Its expression levels increase significantly following focal stroke [6,7], and the antagonism of endothelin receptors can improve stroke [8] and closed head injury (CHI) outcome [9]. It is quite possible that additional, in part unknown, mechanisms are also involved.

The endogenous neuroprotective, restorative brain mechanisms available to the injured brain are based on processes in which adenosine, melatonin and female sex hormones play a role [3,10–11]. Additionally, endogenous antioxidants also contribute towards the brain's ability to cope with post-TBI oxidative stress [12,13]. Constituents, such as VIP-related peptides [14] and others, are also certainly involved.

The endocannabinoid system

Over the past few years the endocannabinoid system has been examined for its neuroprotective role. This system consists of two receptors, CB_1 and CB_2, and three types of endocannabinoid ligands. The CB_1 receptor is present mainly in the central nervous system (CNS) and in numerous peripheral tissues, whereas CB_2 is found mostly in organs of the immune system, but not in the brain [15–18]. Evidence exists for the presence of an as yet unidentified G-protein-coupled cannabinoid receptor in the mouse brain [19]. Arachidonoylethanolamide (anandamide) was the first endocannabinoid to be identified [20], followed by 2-arachidonoyl glycerol (2-AG) [21,22] (Fig. 1). A third endocannabinoid, 2-arachidonyl glyceryl ether (noladin ether), was reported recently [23]. Contrary to the classical neurotransmitters, such as dopamine, serotonin and nor-epinephrine, the endocannabinoid anandamide is present in very low concentrations in the brain and is formed on demand from a precursor, N-arachidonoyl-phosphatidylethanolamine (NAPE) [24], rather than being stored in synaptic vesicles.

Over the past decade, since the discovery of anandamide, this endocannabinoid, as well as 2-AG, have been examined in great detail (reviewed in [25,26]). In most of their pharmacological activities, these body constituents parallel the effects of Δ^9-THC, the active constituent of marijuana. However, due to their rapid cellular uptake and hydrolysis by the specific fatty acid amide hydrolase (FAAH), which surprisingly also affects the ester 2-AG, the time span of the activity of the two endocannabinoids is considerably shorter than that of the plant cannabinoid. A myriad of pharmacological effects of the endocannabinoid are noted in the central and peripheral nervous system [27,29], as well as in the immune [29],

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cardiovascular [30] and reproductive systems [31]. However, the physiological roles played by the endocannabinoids are not yet fully defined. Solid evidence exists that endocannabinoids are involved in amelioration of pain [32], blocking of working memory [33,34], enhancement of appetite [35,36] and suckling [37], cardiovascular modulation [30], possibly mainly during shock, and presumably in preimplantation embryo development and implantation [31]. Using endothelium of human brain capillaries or microvessels we have recently demonstrated the vasorelaxant properties of 2-AG. 2-AG was shown to reduce ET-1-induced Ca^{2+} mobilization, to rearrange the cellular cytoskeleton (actin or vimentin) and to phosphorylate vasodilatory stimulating phosphoprotein [38]. It seems reasonable to assume that the endocannabinoid system is of physiological importance also in psychomotor control [39] and in the regulation of some immune responses [40].

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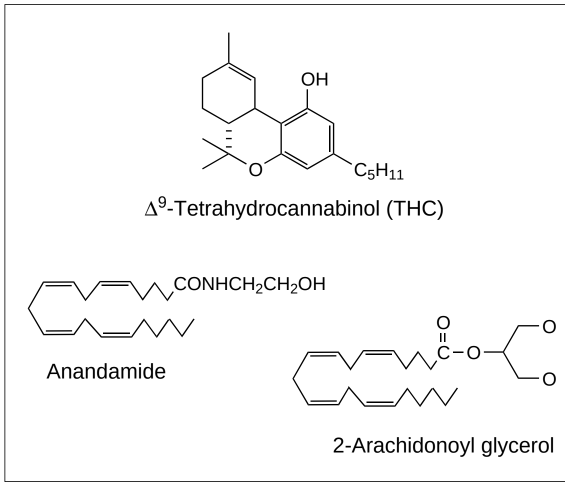

Fig. 1. Structures of Δ^9-tetrahydrocannabinol, the major active constituent in marijuana, and of the major endocannabinoids.

Endocannabinoids and neuroprotection

Here, we would like to summarize the evidence pointing towards a neuroprotective role for endocannabinoids particularly after brain injury. In retrospect it seems that the first experimental evidence that N-acyl-ethanolamines might have a cytoprotective function was reported by Schmid *et al.* [41] who found that such compounds are formed in canine heart following ischemia. The authors speculated that the compounds were formed as part of a protective system, but no further work along these lines was published until anandamide was isolated and identified. In the late 1990s, several independent observations suggested that the endocannabinoids could indeed be cyto- and/or neuroprotectants. Shen *et al.* reported that cannabinoid receptor agonists inhibited glutamatergic synaptic transmission in rat hippocampal cultures [42], and later the same group found that cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity [43]. It was suggested that the protection of the neurons against secondary excitotoxicity was caused by the closing of calcium channels. Hampson *et al.* have noted that anandamide reduces NMDA-induced calcium flux, and that the inhibition is disrupted by a cannabinoid receptor antagonist [44]. The NMDA receptor is a glutamate-sensitive ion channel, associated with excitatory neurotransmission. Recently, Jin *et al.* observed CB_1 cannabinoid receptor induction in experimental stroke [45]. Several groups have noted that although anandamide and its precursor are present in very low, almost undetectable, levels in the brain, their concentrations increase post mortem or on injury [46–48]. Nagayama *et al.* reported that a synthetic CB_1 agonist, WIN-55.212, protected rat brain against ischemia [49] and Sinor *et al.* found that anandamide protects cerebral rat cortical neurons from *in vitro* ischemia [50]. We have reported that 2-AG suppresses formation of reactive oxygen species (ROS) and tumor necrosis factor (TNF)-α by murine macrophages *in vitro* following stimulation with lipopolysaccharide (LPS), and have noted lower levels of TNF-α in serum of LPS-treated mice after administration of 2-AG [51]. Both classes of mediators, ROS and TNF-α, are major contributors to pathophysiology of brain injury. Van der Stelt *et al.* recently reported that Δ^9-THC protects rat brain against ouabain-induced excitotoxicity [52]. As mentioned above, we have shown that 2-AG affects the assembly of cytoskeleton filaments and counteracts the vasoconstrictory effects of ET-1 [38]. It therefore might protect from the ischemic episode that occurs after TBI, and thus exert a cerebroprotection effect after brain injury [53]. Together, these data strongly suggest that the endocannabinoid system is intimately involved in neuroprotection.

Evidence that endocannabinoids are neuroprotective *in vivo*

Recently, several groups reported novel, though in part contradictory, *in vivo* results with anandamide and 2-AG. Hansen *et al.* found that in response to injury high levels of the anandamide phospholipid precursor are produced in rat neonatal brain and that 24 h after a mild concussive head trauma in young rats anandamide levels increased about three fold, with no concomitant increase in 2-AG formation [54]. By contrast, our group found that after severe closed head injury (CHI) in mice, the level of 2-AG was

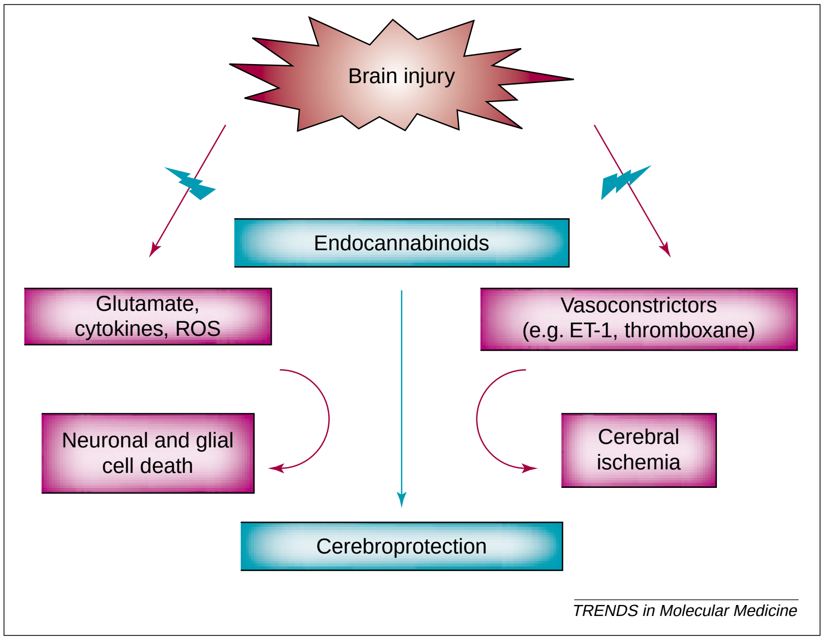

Fig. 2. Mechanisms of cerebroprotection by endocannabinoids. Brain injury triggers the release of harmful mediators such as glutamate, cytokines and reactive oxygen species (ROS), which in turn induce neuronal and glial cell death. Vasoconstrictors, such as endothelin (ET) and thromboxane, are also elevated after brain injury and contribute, by acting locally at the cerebral vasculature, to the development of ischemia. Endocannabinoids, which can be also elevated after trauma, inhibit the release of glutamate, ROS and cytokines, as well as the activity of ET. All these mechanisms of endocannabinoids contribute towards cerebroprotection.

significantly elevated [55]. Similarly, Sugiura *et al.* have recorded that 2-AG levels are enhanced in rat brain after picrotoxinin administration or decapitation [56]. To test the role of 2-AG we administered synthetic 2-AG to mice after CHI, and found significant reduction of brain edema, better clinical recovery and reduced infarct volume compared to controls [55]. Histological data strongly supported the above observations. When 2-AG was administered together with additional 2-acyl-glycerols, that are present in the brain, but have no protective activity of their own, functional recovery was significantly enhanced [55]. The beneficial effect of 2-AG was dose-dependently attenuated by SR141716A, an antagonist of the CB_1 cannabinoid receptor. These results indicate that in mouse brain after injury the endocannabinoid 2-AG might play a neuroprotective role in which the cannabinoid system is involved [55]. Van der Stelt *et al.* showed in a longitudinal magnetic resonance imaging (MRI) study that anandamide, like Δ^9-THC, reduces neuronal injury in a dose-dependent manner in a rat model of ouabain-induced excitotoxicity [57]. Already 15 min after ouabain injection, neonatal rats treated with anandamide had a 47% smaller volume of cytotoxic edema than vehicle-treated animals. After seven days, the anandamide-treated animals had a 67% smaller infarct. Application of the CB_1-receptor antagonist SR141716 alone did not increase the infarct size, arguing against a CB_1-mediated protective role of endogenously released endocannabinoids in this system. A preliminary GC-MS study to quantify endocannabinoid levels in neonatal rat brain after ouabain injection did not show a significant increase in either anandamide or 2-AG [57]. Figure 2 depicts some routes through which endocannabinoids might affect brain injury.

The above, partly contradictory, results can be rationalized if it is assumed that both anandamide and 2-AG are produced on brain trauma, be it either chemical or mechanical, however, the production of a specific endocannabinoid might depend on the species (mouse or rat), age, severity of the trauma and type of injury.

Neuroprotection by non-psychotropic cannabinoids

A synthetic, non-psychotropic cannabinoid HU-211 (Dexanabinol) is in phase III clinical trials against brain trauma [58]. This compound was found to exhibit pharmacological properties characteristic of a NMDA (glutaminergic) – receptor antagonist. It blocks NMDA receptors by interacting with a site close to, but distinct from, that of uncompetitive NMDA antagonists. HU-211 also blocks TNF-α synthesis and has antioxidant properties. It protects cultured neurons from the toxic effects of reactive oxygen species (ROS). Since glutamate, ROS and TNF-α are well known to be implicated in the pathophysiology of brain injury, the above observations led to clinical trials which have shown that HU-211 significantly improves the neurological outcome of head injured patients. Hampson *et al.* have found that, like HU-211, the cannabis constituent cannabidiol, which does not bind to the cannabinoid receptors, is a potent antioxidant and reduces glutamate toxicity [59].

As endocannabinoids are rapidly inactivated by cellular uptake followed by hydrolysis [60,61], enhancement of their neuroprotective activity could possibly be achieved by impairment of their inactivation. Indeed numerous compounds are known to block cellular uptake and/or hydrolysis [60,61]. However this obvious route has not been followed (or possibly not yet reported) so far in investigations on neuroprotection with cannabinoids.

Conclusions

Anandamide and 2-AG seem to be endogenous neuroprotective agents produced by the brain, and presumably by other neuronal systems, on trauma. The cannabis plant constituent, Δ^9-THC, which is a cannabinoid receptor agonist, is also neuroprotective. Cannabidiol, which does not bind to the cannabinoid receptors, also reduces glutamate toxicity. A synthetic cannabinoid HU-211, which does not bind to cannabinoid receptors, is in clinical trials against the neurological damage of brain trauma. The mechanisms of neuroprotection by the various plant, synthetic and endogenous cannabinoids are not yet clear. In some cases it has been shown that cannabinoid receptors are involved, however the neuroprotective effects of compounds that do not bind to the cannabinoid receptors or are not antagonized by CB_1 receptor antagonists point out that non-cannabinoid receptor mechanisms are also involved. We expect that within the next few years these mechanisms will be clarified and cannabinoid-based drugs for brain trauma will be introduced in the clinic.

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