Published in final edited form as:
Curr Pharm Des. 2008 ; 14(23): 2347–2361.

Role of cannabinoids and endocannabinoids in cerebral ischemia

Cecilia J. Hillard
Department of Pharmacology and Toxicology; Medical College of Wisconsin; Milwaukee, WI USA 53226

Abstract

The human costs of stroke are very large and growing; it is the third largest cause of death in the United States and survivors are often faced with loss of ability to function independently. There is a large need for therapeutic approaches that act to protect neurons from the injury produced by ischemia and reperfusion. The goal of this review is to introduce and discuss the available data that endogenous cannabinoid signaling is altered during ischemia and that it contributes to the consequences of ischemia-induced injury. Overall, the available data suggest that inhibition of CB1 receptor activation together with increased CB2 receptor activation produces beneficial effects.

Keywords

CB1 cannabinoid receptor; CB2 cannabinoid receptor; rimonabant; fatty acid amide hydrolase; N-arachidonylethanolamine; anandamide; 2-arachidonoylglycerol

INTRODUCTION

Interruption of cerebral blood flow, regardless of the cause, results in a reduction of nutrient delivery to the brain ultimately leading to ischemic cell death [1,2]. In the majority of cases of human stroke, the reduction in cerebral blood flow is transient and cell death does not occur immediately. The delay between the initial insult and the cellular damage is variable, from hours to days or even weeks, depending upon the nature of the insult and the brain region affected. An important implication of this delay is that the introduction into the brain of protective therapeutic agents during this critical period could disrupt the cascade of damaging events and salvage at-risk brain tissue. These therapeutic agents are defined as “neuroprotective” and many have been explored in animal and human studies. There is evidence that endogenous homeostatic mechanisms are evoked, with both positive and negative consequences, that can be manipulated to reduce the likelihood of cell death.

Another important aspect of human stroke is the spatial heterogeneity of cellular injury. Neurons in the core of the ischemic region (defined as receiving less than 15% of normal blood flow) can be differentiated from neurons in the penumbral region, where blood flow is less than 40% of normal during the ischemia [1]. A third region can also be defined as the extrapenumbral or peri-infarct region that receives between 40 and 100% of normal blood flow. There are major differences in the biochemical changes that occur in each of these regions; importantly, the differences are reflected as changes in susceptibility to cell death and in neuroprotection.

Address correspondence to: Cecilia J. Hillard, Ph.D. Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226 USA, Phone: 414-456-8493, Fax: 414-456-6545, Email: chillard@mcw.edu.

The consequences of interruption or cessation of cerebral blood flow are dependent upon many factors; including whether the cessation in blood flow is transient or permanent; focal or global. Considerable progress has been made in understanding of the underlying causes of cell death that occur as a result of ischemia through the use of in vivo animal models as well as neuronal cell culture approaches. Loss of oxygen and glucose that accompany a cessation of blood flow rapidly initiates significant changes in intracellular ion and metabolite homeostasis. Inhibitor studies indicate that the generation of free radicals and a maintained increase in intraneuronal calcium and sodium are among the most damaging consequences of a short period of global ischemia [1]. These changes lead to both necrotic and apoptotic cell death, which occur in parallel and likely potentiate each other. In addition to a prolonged accumulation of free radicals and intracellular calcium and sodium, decreased pH and increased nitric oxide generation (NO) contribute to the size of the infarct following a permanent, focal ischemic episode. It is thought that release of glutamate from the core of the infarcted area leads to a prolonged increase in intracellular calcium via activation of ionotropic, glutamate receptors in the penumbral region. In support of this idea, agents that inhibit calcium influx through N-methyl-D-aspartate (NMDA) receptors are generally protective against cell death resulting from focal ischemia [3]. During transient episodes of ischemia, reperfusion occurs and other mediators of neuronal injury come into play, including the activation of poly-(ADP)-ribose polymerase (PARP-1); stress kinase signaling and the recruitment of leukocytes into the area of injury followed by neuroinflammation [2]. Therefore, multiple mechanisms are involved in the overall response of the brain to ischemia and the relative contributions of these mechanisms to neuronal death varies depending upon the model systems employed.

The endocannabinoid signaling system (ECS) has several features that support its hypothesized involvement in ischemic injury (Figure 1). First, the endocannabinoids (eCBs) and related lipids accumulate in ischemic tissues [4,5] supporting the hypothesis that the ECS is activated during ischemia. Second, accumulating data support a general role for ECS in the maintenance of metabolic homeostasis [6] and responsivity of the brain to stress [7]. Third, activation of the CB1 cannabinoid receptor results a decrease in the probability of opening of voltage-operated calcium channels [8–10] which could result in a reduction in intraneuronal calcium contents. In addition, a subset of CB1 receptors are present on glutamatergic nerve terminals and their activation results in inhibition of glutamate release in response to depolarization [11]. Together these signaling mechanisms are consistent with neuroprotection because they lead to decreased intracellular calcium. Fourth, the CB2 cannabinoid receptor is expressed by immune cells [12], including brain resident microglial cells [13], and its activation results in a decrease in the release of pro-inflammatory mediators [14]. Fifth, the CB1 cannabinoid receptor is present in the cerebral vasculature [15–17] and its activation produces vasodilation [15,18,19]. Importantly, data suggest that activation of cerebrovascular CB1 receptors results in loss of autoregulation [20], which could exacerbate the effects of ischemia.

To summarize, both theoretical considerations and experimental data suggest that the ECS contributes to the consequences of cerebral ischemia via multiple mechanisms. However, the role of the ECS is complex, and it can both exacerbate and reduce injury. The purpose of this review is to discuss the available data describing the effects of cerebral ischemia on the various components of the ECS and the role of the ECS in the consequences of cerebral ischemia.

EFFECTS OF ISCHEMIA ON COMPONENTS OF THE ECS IN BRAIN

1. Changes in brain endocannabinoid content

Two arachidonic acid derivatives, *N*-arachidonylethanolamine (AEA or anandamide) and 2-arachidonoylglycerol (2-AG) function as endogenous ligands of the cannabinoid receptors (see [21] for a review of the biochemistry of the eCBs). The earliest studies of the family of the *N*-acylethanolamines (NAEs), of which AEA is a minor constituent, demonstrated that members

of this family accumulate in tissues deprived of blood flow. In particular, the NAEs accumulate in post-mortem brain [5,22–24] as do their immediate precursors, the *N*-acylphosphatidylethanolamines (NAPEs) [25,26]. Saturated and mono-unsaturated NAEs (such as palmitoylethanolamide (PEA) and oleolyethanolamide (OEA)) are present at much higher concentrations than AEA in the brain immediately after death and they accumulate in a manner that is linearly dependent upon time post mortem [5,24]. On the other hand, although AEA contents are very low in freshly isolated tissues, AEA exhibits an exponential increase post-mortally [5,24]. Studies using inhibitors of the major catabolic pathway of the NAE family of lipids, fatty acid amide hydrolase (FAAH) [27], as well as FAAH null mice surprisingly demonstrated that the exponential accumulation of AEA is dependent upon FAAH activity [24]. Further studies suggest that the accumulation of AEA is the result of non-enzymatic condensation of ethanolamine generated by FAAH-mediated catabolism of the NAEs with arachidonate-containing phospholipids, a process that has been observed to occur in vitro as well [23].

Post mortal changes in brain 2-AG content have not been as consistently observed. Sugiura and colleagues have reported that 2-AG increases in rat brain within the first minute after decapitation, and decreases back to baseline concentrations at 5 min [28]. However, the only way to observe this change is to freeze the brain in place immediately after death, a procedure that is not generally employed and standard methods of brain harvest miss this change in 2-AG content [24].

Several studies have examined the effects of controlled interruptions in cerebral blood flow on tissue eCB contents. NAE contents have been examined in brain tissue following MCA occlusion (without reperfusion) of various durations in rats [29–31]. A five hour occlusion of the rat MCA increased the contents of 6, long chain NAEs in the striatum ipsilateral to the occlusion, including a 23-fold increase in PEA and a 12-fold increase in AEA [29]. Similar, although smaller, changes were seen in the cerebral cortex, a brain region that is less sensitive to interruptions in blood flow through the MCA [29]. The NAEs likely accumulate earlier in the ischemic period as AEA content in the ipsilateral hemisphere was significantly increased in tissue harvested immediately following a 30 min MCA occlusion [30] and in the ipsilateral striatum immediately following a 2 hour MCA occlusion [31] The increase in AEA in these studies was much less than seen after 5 hours, which is consistent with the post-mortem data demonstrating that the NAEs, including AEA, continually accumulate. Therefore, it appears that increasing the duration of the occlusion both increases the amount of AEA that accumulates in the ischemic compared to the non-ischemic hemisphere and the spread of regions in which an increase can be detected. No changes in 2-AG content in the ischemic hemisphere were seen in rats after a 30, 60 or 120 min MCA occlusion [30].

Several studies have explored the biochemical mechanisms involved in the effects of ischemia on tissue NAE contents. Berger and colleagues reported that 5 hours of MCA occlusion in rats produced relatively small, significant increases in the NAPE precursors for the saturated family members (PEA and stearoylethanolamide, SEA) but not for the unsaturated species [29]. These findings suggest that increased biosynthesis, at the level of precursor generation, could play a role in the increase of at least some NAEs following ischemia in rats. In addition, NAPE hydrolysis was shown to be greater in membranes harvested from the ischemic than non-ischemic striatum after a 2 hour occlusion of the MCA [31], suggesting that the flux through the biosynthetic pathway for the NAEs is increased in ischemic tissue. On the other hand, the increase in AEA content in the ischemic striatum following 2 hour MCA occlusion was accompanied by a decrease in the protein content and activity of FAAH [31]. These data suggest that increased accumulation of AEA could also be driven by a reduction in its catabolism in the ischemic region. The finding that ischemia results in a combination of an increase in NAPE hydrolysis together with a decrease in FAAH expression is consistent with the data that

ischemia results in greater relative increases in the amounts of the NAE family members than of their NAPE precursors [29]. In sum, the available data indicate that the accumulation of the NAEs following ischemia in rats is driven by a combination of biochemical changes, including: increased precursor generation; increased rate of conversion of the NAPEs to NAEs; and decreased catabolism of the NAEs.

Somewhat different results were obtained following permanent occlusion of the MCA in mice [32] which call into question the generalizability of the findings in rats. As in rats, MCA occlusion produced an increase in the NAEs, including PEA and SEA in the hemisphere ipsilateral to the MCA occlusion. However, in contrast to the findings in rats, no significant change in AEA was seen after 4, 12 or 24 hours of MCA occlusion. On the other hand, 2-AG content was significantly increased in the ipsilateral hemisphere after 4, but not 12 or 24, hours of MCA occlusion. Also in contrast to the results obtained in rats, the increase in NAEs in mice was not accompanied by an increase in NAPE-PLD expression or a change in the expression or activity of FAAH. Therefore, it is possible that the increase in NAEs seen in mice is driven by increased NAPE synthesis or an increased NAPE hydrolytic activity in vivo that does not persist ex vivo (due to increased calcium in vivo, for example). The reasons for the differences in the response to ischemia between rats and mice are currently not evident and should be explored in the future. However, it is striking that ischemia results in a significant accumulation of the NAEs in both species even though the mechanisms differ.

Results of studies in which the effects of ischemia on two prototypical NAEs, PEA and AEA, are compared show that the relative increase in PEA is much greater than the relative increase in AEA. These results suggest that ischemia exerts a selective increase in saturated rather than polyunsaturated NAEs compared to their abundance in the uninjured brain. In rats, this is seen as a reduced relative accumulation of AEA compared to PEA and in mice it is seen as a lack of AEA accumulation in spite of a 40-fold increase in PEA while in mice, PEA is increased without a change in AEA. This finding is somewhat surprising since, for the most part, the same pathways for the biosynthesis and catabolism are operative for all of the NAE family members [33] which would lead to the expectation that relative changes in all of the NAE species should be similar. The selectivity could lie in the mechanism for the synthesis of the NAPEs, immediate precursors for the NAEs as the study by Berger and colleagues discussed above found that 5 hours of MCA occlusion increased the tissue concentrations of NAPE precursors for saturated NAEs (PEA and SEA) but not for AEA [29]. In fact, the content of N-arachidonyl-PE in the ischemic cortex was significantly less than in the contralateral cortex. These findings suggest that some of the difference in the relative accumulation of the NAE family members could result from substrate selectivity at the step of NAPE synthesis. NAPE synthesis is thought to occur through the action of N-acyltransferase (NAT) enzyme(s) that add acyl groups to the ethanolamine moiety of phosphatidylethanolamine [33]. Interestingly, NAT activity is calcium-sensitive and has been hypothesized to be the rate-limiting step in the biosynthesis of the NAEs. Recent data suggest that several enzymes function in the brain as NATs with different substrate specificities and, importantly, differential sensitivities to regulation by calcium [34]. However, the NAT activity involved in the synthesis of the precursor for AEA was more sensitive to changes in calcium than the activity involved in the synthesis of the precursor for PEA, which would result in the opposite finding (i.e. greater relative accumulation of AEA than PEA). It is also possible that differences in the catabolism of the NAEs contributes to the differential accumulation. In particular, an amidase that hydrolyzes the N-acylethanolamines and is not FAAH has been identified which has preference for PEA over AEA; has an acidic pH optimum; and is expressed by macrophages [35] Although not tested, it is possible that this enzyme, called N-acylethanolamine acid amidase, could be an important regulator of NAE content in the neuro-inflamed brain in particular. However, the fact that this enzyme has preference for PEA over AEA and PEA accumulates more than AEA during ischemia argues against this possibility.

Recent studies [31] indicate that administration of the neuroprotectant, estrogen (0.2 mg/kg, i.p.) to male rats 1 hour before the MCA occlusion, abolished the increase in striatal AEA. This effect of estrogen was antagonized by ICI182 780, suggesting that the intracellular estrogen receptor (ER) is the site of action of estrogen in this effect. Estrogen treatment significantly increased FAAH activity and decreased NAPE hydrolysis, both of which are consistent mechanistically with a decrease in AEA content. Interestingly, FAAH expression has been shown to be regulated by estrogen in other studies [36–38]; with both increases and decreases in expression occurring. The mechanisms involved in the regulation of FAAH by estrogen and other steroids is an important topic that needs more experimental attention, particularly since it is well known that estrogen is protective against stroke [39].

While there are some data regarding the biochemical mechanisms involved in increased tissue NAE following ischemia, only a few studies have investigated the specific triggers involved in its accumulation. As was mentioned above, data indicate that an increase in tissue AEA content is a very early event in the cascade of changes that occurs during ischemia; in particular, AEA is significantly increased following 30 min of MCA occlusion [30]. Berger and colleagues demonstrated that the effect of focal ischemia on tissue NAE contents was not affected by pre-treatment of rats with the NMDA receptor blocker, MK-801 at a neuroprotective dose [29]. These findings suggest that the increase in tissue NAE is upstream of NMDA activation during ischemia. This finding is important in light of data from neurons in culture that activation of NMDA receptors results in a robust increase in NAE, including AEA, production [40]. Taken together, it appears that the regulation of tissue NAE content is multi-factorial and can occur by mechanisms other than NMDA receptor over-activation and, thus, in the absence of glutamate-mediated excitotoxicity.

Two studies have examined the effects of ischemia followed by reperfusion on brain NAEs. Both studies found that the NAEs were increased after reperfusion and suggest that the addition of a reperfusion period following ischemia results in exacerbation NAE accumulation compared to ischemia alone. In a study carried out in mice by Stella and colleagues, one MCA was occluded for 20 min followed by 24 hours of reperfusion. This ischemia/reperfusion procedure resulted in a 25-fold increase in PEA; 3-fold increase in AEA and 4-fold increase in docosatetraenoylethanolamide (DEA) in the cerebral cortex compared to sham-operated controls [41]. Maccarrone, Bagetta and colleagues have reported that brain AEA contents are increased more during reperfusion following MCA occlusion than during occlusion alone in rats [31]. When a 2 hour occlusion is followed by 1, 6 or 22 hours of reperfusion, striatal AEA contents were increased significantly compared to those at the end of the occlusion. On the other hand, AEA content in the cerebral cortex was unaffected after 1 hour of reperfusion and was significantly decreased at 6 and 22 hours after reperfusion. The expression of FAAH in the striatum was not different between rats exposed to MCA occlusion alone and those exposed to occlusion followed by 1 hour of reperfusion; however, NAPE hydrolysis in the striatum was significantly increased in the rats that were reperfused. These studies suggest that two mechanisms are involved in the accumulation of AEA during a transient, ischemic episode. Tissue contents of the NAEs increase as a result of the ischemia per se possibly due to a combination of increased synthesis and decreased degradation as described above; AEA is either the least affected NAE or not affected at all as discussed. Changes that occur after the re-establishment of blood flow to the ischemic region also promote NAE, and specifically AEA, accumulation, perhaps via increased synthetic capacity. A single case study in a patient with a hemispheric stroke supports the rodent data in that AEA, PEA and OEA contents were increased in microdialysates of tissue surrounding the ischemic lesion [42].

Changes in the brain content of 2-AG in response to ischemia have been examined in a few studies. No changes in 2-AG content in the ischemic hemisphere were seen in rats after a 30, 60 or 120 min MCA occlusion [30]. Interestingly, mice exposed to a sham surgery (which

involved anesthesia and incisions but no vessel occlusions) exhibited significant increases in 2-AG content in the cerebral cortex compared to untreated controls 24 hours after the intervention [41]. Mice in which the MCA was occluded had lower 2-AG contents than the sham surgery animals. These studies raise the intriguing possibility that one or more of the aspects of the surgical intervention itself (e.g. anesthesia or activation of stress responses) increases tissue 2-AG while ischemia itself actually decreases 2-AG. Other types of brain injury have been shown to affect this eCB. For example, 2-AG accumulation is seen within minutes of the injection adult rats with the GABA antagonist, picrotoxinin, at doses that induce seizures [43]. In addition, 2-AG accumulates in the injured hemisphere following a concussive, closed head injury in mice [44] and accumulates in the spinal cord, together with AEA, in a mutant mouse line with chronic spinal cord degeneration [45].

Although much remains to be elucidated particularly regarding the mechanisms involved, the available data support the hypothesis that ECS is activated at the level of the endocannabinoid, AEA, during ischemia and reperfusion. Although AEA content is significantly increased by ischemia, it is dwarfed by increases in other members of the NAE family, particularly PEA. 2-AG contents in the brain appear to unaffected (or possibly decreased) by changes in blood flow but are altered in response to physical trauma, seizures, and/or anesthesia.

As was discussed in the introduction, ischemia and reperfusion produce neuronal death through multiple mechanisms, including glutamate-mediated excitotoxicity and neuroinflammation. Excessive activation of glutamatergic signaling as occurs during seizures or excitotoxicity has also been shown to increase eCB contents in brain. Both NAPE and NAE concentrations increase in neonatal rats 4 and 24 hours after intracranial injection of NMDA [46]. In contrast with the changes that occur during ischemia (described above [29]), the NAPE species increased to a greater extent than the NAEs after NMDA injection [46] suggesting that the mechanisms involved in NAE accumulation are not identical in ischemia and excitotoxicity. Interestingly, intracranial ouabain administration into neonatal rats, which produces neurotoxicity that can be partially blocked by NMDA receptor antagonism [47], did not affect brain AEA or 2-AG concentrations at 2, 8 or 20 hours after injection [48].

2. Changes in brain cannabinoid receptor expression

There have been several studies exploring the hypothesis that ischemia alters the amount of either of the two established cannabinoid receptors (CB1 and CB2) in the brain. In the first study to tackle this question, Greenberg and colleagues used transient MCA occlusion in rats to induce ischemia/reperfusion injury [49]. They found that CB1 receptor-like immunoreactivity was up-regulated in neuronal-type cells in the ischemic boundary zone as early as 2 hours after reperfusion. There were no changes in CB1 receptor-like immunoreactivity in the ischemic core of the infarct. The antibody used for this study was from a commercial source and its specificity for the CB1 receptor protein was not addressed in the study. However, these results are consistent with those of a recent study in which quantitative PCR was used to measure CB1 receptor mRNA content following transient, MCA occlusion in mice [50]. The mRNA for the CB1 receptor in the brain ipsilateral to the occlusion was significantly elevated at 1 hour, and maximally elevated 6 hours, after the ischemia. In contrast, 5 hours of permanent MCA occlusion had no effect on CB1 receptor binding site density in male rats [51]. Therefore, it is possible that the increase in CB1 receptor following transient ischemia is a results of reperfusion rather than ischemia per se. A mild, concussive trauma in neonatal rats also results in a small but significant increase in CB1 receptor binding site density and mRNA [52]. In contrast, excitotoxicity evoked by NMDA injections into rat pup striatum resulted in a decrease in CB1 receptor ligand binding and mRNA expression in the striatum and adjacent cortical regions 4 hours after the injection [52]. Taken together, these studies suggest that reperfusion injury is associated with a rapid increase in CB1 receptor expression,

possibly driven by increased mRNA. The mechanism involved in the increased expression is not clear, but it seems not be associated with excitotoxicity.

In the global ischemia model in gerbils, 5 min of ischemia followed by 24 or 48 hours of reperfusion did not affect CB1 receptor protein or binding site density in the hippocampus, the brain region most affected by this intervention [53]. It is possible that CB1 receptor changes only occur following focal ischemia/reperfusion; or the CB1 receptor expression did change in the gerbil study, but it occurred at an earlier time point and was not detected in this study.

It is known that exposure of gerbils to a short period of global ischemia (2.5 min) reduces neuronal death in response to a subsequent, longer ischemic episode. This phenomenon is called preconditioning and it is a model of ischemia tolerance induction [54]. In contrast to the lack of effect of a 5 min ischemic period, 2.5 min of global ischemia produced a significant reduction in CB1 receptor immunoreactivity and binding site density in both the CA1 and CA3 regions of the hippocampus at 24, 48 and 96 hours after the ischemia. This is an extremely interesting observation and it suggests the hypothesis that decreased CB1 receptor activation in the area at risk for infarction is protective. On the other hand, another study suggested the opposite conclusion since feeding of mice a calorie restricted diet resulted in an increase in CB1 receptor immunoreactivity in the striatum and hypothalamus and protected against ischemia [55]. However, both of these studies are correlative and more data is clearly required before conclusions regarding the role of CB1 receptor mediated signaling in ischemic injury are made.

The CB2 cannabinoid receptor is found on circulating immune cells [12] and at low levels in perivascular microglia in the uninjured brain [56]. However, during states of neuro-inflammation, CB2 receptor expression in the brain is increased in brain resident microglial cells [14,57] and via migration into the brain of macrophages [13] and T cells [58] which express CB2 constitutively. Ashton and coworkers have recently reported that CB2 receptor expression is increased in two models of ischemia [59]. In 26 day old rats exposed to hypoxia and ischemia 3 days before, CB2 receptor-positive cells were seen on the lesioned side of the brain. The CB2-positive cells were co-localized with areas of reactive astrocytosis and had a macrophage-like phenotype. Similarly, CB2 receptor-positive, macrophage or microglial cells were seen in the ischemic penumbra of the cortex 3 days following transient MCA occlusion in adult rats. In another study of the effects of transient MCA occlusion in mice, CB2 receptor mRNA expression was found to be decreased in the ischemic hemisphere 1 and 3 hours after the ischemia and significantly increased 24 hours after. These studies suggest that CB2 expression increases later in the time course of the ischemia, which is consistent with its presence on macrophages or leukocytes recruited into the injured brain. Although studies of the effects of ischemic injury on CB2 receptor expression in brain are limited, the results thus far are consistent with a general effect of neuro-inflammation to increase the presence in brain of cells expressing CB2 receptors [60].

EFFECTS OF ALTERATIONS IN THE ECS ON THE OUTCOME OF ISCHEMIA/ REPERFUSION INJURY

1. Evidence that increased CB1 receptor activation is protective

There are several in vivo studies that support the hypothesis that CB1 receptor activation prevents neuronal death in response to ischemia. Male rats exposed to 15 min of global ischemia followed by reperfusion exhibit a profound neuronal loss in the CA1 region of the hippocampus 24 hours later [61]. Pretreatment of the rats prior to the ischemia with the CB1 receptor agonist, Win 55212-2, produced a dose-related and significant increase in the number of neurons surviving; the protection induced by Win 55212-2 was completely antagonized by the CB1

receptor antagonist rimonabant (also called SR141716 and SR141716A). Similarly, CB1 receptor agonists have also been shown to reduce infarct volumes following permanent MCA occlusion. Win 55212-2 produced a significant reduction in infarct volume when administered either 30 min before or 30 min after the onset of occlusion but not when administered more than 60 min after occlusion [61]. This effect was also completely reversed by rimonabant. Several other studies using different compounds confirm that exogenous administration of CB1 agonists before, during, or immediately after the onset of a permanent, focal ischemic event reduce the amount of neuronal damage [62–64]; although none of these studies demonstrated convincingly that the CB1 receptor was involved. CP55940 was found to protect against EEG and motor activity changes induced by a 10 min, bilateral occlusion of the carotid arteries in gerbils [65]. In that study, CP55940 was administered 5 min after the reperfusion and produced a long-lasting (at least 7 day) protection that was antagonized by rimonabant. These studies support the hypothesis that activation of the CB1 receptor by highly efficacious, exogenous agonists during the acute phase of ischemia decreases the likelihood of the occurrence of a detrimental event at the time of ischemia and thereby reduces the amount of infarction and neuronal death long-term.

A protective role of the CB1 receptor is also supported by studies in CB1 receptor null mice [66]. The knock out mice demonstrated increased mortality from permanent MCA occlusion as well as increased infarct size and neurological deficits following transient MCA occlusion. Interestingly, the lack of CB1 receptors also resulted in a more severe ischemia in that cerebral blood flow to the ischemic penumbra during reperfusion was reduced, suggesting that CB1 receptor-regulation of cerebral vessels are involved in the protective effects.

As was discussed in the section above, in vivo data demonstrate that ischemia increases brain content of the eCBs. Since the presence of CB1 agonists in the brain during ischemia results in reduced injury, these finding have lead to the hypothesis that the ECS functions as an endogenous, neuroprotective mechanism. A prediction of this hypothesis is that blockade of the CB1 receptor during ischemia will exacerbate ischemia-induced neurotoxicity. The data obtained in the CB1 receptor null mice that ischemia is more severe support this hypothesis [66]. On the other hand, pharmacological blockade of the CB1 receptor with rimonabant during ischemia did not worsen the neurotoxicity produced by global or focal ischemic in rats [61]; or gerbils [65].

Another prediction of the endogenous mediator of neuroprotection hypothesis is that treatments that elevate the eCBs during the ischemia should be protective. Support for this hypothesis comes from studies of Degn and colleagues who showed that pretreatment of mice with an inhibitor of FAAH resulted in a significant decrease in infarct volume 24 hours later [32]. However, these investigators could not detect an increase in eCB content in response to the FAAH inhibitor and did not examine the role of the CB1 receptor in the neuroprotective effect of the inhibitor. Given that FAAH is the primary catabolic enzyme for the entire family of NAEs [67], it is possible that the neuroprotection is mediated by a family member without CB1 receptor activity. Another study found that AM404, an indirect agonist of the CB1 receptor [68], is protective against neuronal damage induced by transient, global ischemia in the gerbil [69]. However, antagonist studies suggest that some but not all of the protective effects of AM404 are CB1 receptor dependent. Interestingly, the protective effects of AM404 (and THC) in this model were reversed by the opiate receptor antagonist, naloxone. Finally, there is other evidence (discussed further below) demonstrating that inhibition of the CB1 receptor during ischemia can be protective. Therefore, the hypothesis that activation of the ECS occurs during ischemia and functions as a neuroprotective mechanism via activation of the CB1 receptor is not well supported by the available data (Table 1). While aspects of this hypothesis are supported, the ECS is more complex and, in specific, cannot be considered a single entity in the context of ischemia.

2. Mechanisms involved in the protective effects of CB1 receptor activation

a. CB1 receptor activation reduces excitotoxicity—As described above, several in vivo studies have demonstrated that exogenous activation of the CB1 receptor during ischemia is neuroprotective. There have been several hypotheses put forward to explain this protection at a mechanistic level. The first hypothesis is that CB1 receptor activation protects against glutamate-induced excitotoxicity. This hypothesis is supported by the effects of CB1 agonists in cell culture models; but there seem to be at least two different mechanisms by which this occurs. CB1 receptor activation reduces excitotoxicity in cultured hippocampal neurons when toxicity is induced by magnesium removal [70,71]. CB1 receptor agonists did not protect against cell toxicity produced by application of glutamate in hippocampal neurons, suggesting that their protective effect was presynaptic and secondary to inhibition of glutamate release. This finding is consistent with the nearly exclusive distribution of CB1 receptors on presynaptic terminals in the hippocampus [72]. Cell death induced by magnesium removal is due to a sustained, repetitive pattern of calcium spiking that is dependent upon NMDA receptor activation. The protective effect is likely due to the ability of presynaptic CB1 receptor activation to hyperpolarize the neuronal membrane [73] and directly inhibit the opening of voltage-operated calcium channels [9]. This mechanism would result in decreased calcium spiking directly (through inhibition of influx through voltage operated channels) and indirectly, via inhibition glutamate release. CB1-receptor mediated decrease in calcium influx through voltage operated calcium channels results in reduced activity of neuronal nitric oxide synthase (nNOS) [74]. Since nitric oxide is thought to contribute to the detrimental effects of ischemic injury [2], this mechanism could also contribute to the beneficial effects of CB1 receptor agonists.

Other studies indicate that the CB1 receptor also reduces the deleterious effects of excessive glutamate by effects that are down-stream of glutamate receptor activation. The first evidence for this mechanism came from spinal cord cultures in which CB1 receptor activation was protective against neuronal death evoked by the direct application of kainic acid [75]. The possibility that this mechanism is endogenously active comes from studies demonstrating that kainic acid produces excessive excitation in cultured CA1 hippocampal neurons null for the CB1 receptor compared to wild type neurons [76] and in hippocampal neurons exposed to rimonabant [77]. Since kainic acid treatment of hippocampal slices results in an increase in NAPEs [78], these findings suggest that the ECS is an endogenous neuroprotective mechanism that is recruited by activation of the kainate subtype of glutamate receptors. However, a protective effect of CB1 agonist against glutamate-mediated toxicity in neuronal cultures has not been seen in all studies in which it was examined [70,79]. It is possible that the differential findings are related to cAMP signaling as one study found that the CB1 agonist, CP55940, was protective only if cellular cAMP concentrations were elevated [80].

A recent study using the hippocampal slice used changes in glutamatergic signaling (rather than cell death) as an end point and provided some evidence that CB1 receptor activation contributes to decreased glutamatergic signaling that occurs following oxygen and glucose deprivation (OGD) [81]. The transient decrease in glutamate release is thought to be a protective mechanism since reduced glutamate will result in decreased excitotoxicity. The outcome measured in this study was depression of excitatory post synaptic potentials (EPSPs), a transient and early change induced by nutrient deprivation. The CB1 receptor agonist, Win 55212-2, reduced glutamate transmission in control slices but HU 210 did not. Win 55212-2 had no additional effect to depress EPSPs in slices exposed to OGD. These results could support the hypothesis that ODG has induced eCB release and the eCBs maximally activate the CB1 receptors on glutamatergic terminals to suppress glutamate release, thus abrogating the effect of Win 55212-2. While CB1 receptor blockade did not reverse the effect of ODG to suppress EPSPs, AM251 increased the recovery of normal transmission following the insult. The authors

interpret these data as support for a role for the ECS in protection from ODG by transiently decreasing glutamate release and thus NMDA receptor activation.

These in vitro studies are supported by in vivo studies demonstrating that activation of the CB1 receptor is protective in models of excitotoxic injury. The marijuana derived CB1 agonist, Δ^9-tetrahydrocannabinol (THC) reduces neuronal injury in neonatal rats injected with ouabain [82]. Ouabain, which inhibits Na-K ATPase, results in secondary excitotoxicity. THC reduced both the volume of cytotoxic edema acutely and decreased the amount of neuronal damage 7 days after ouabain treatment. These effects of THC were prevented by co-administration of rimonabant. In a second study using the same model, AEA was also found to be protective in the acute phase via a non-CB1 receptor mechanism [48] and at 7 days via a CB1 receptor mechanism [83]. AEA also protects against kainate-AMPA toxicity in newborn rodents; this effect is blocked by a very high dose of the CB1 receptor antagonist AM251 and mimicked by one but not another CB1 agonist leaving the question of the role of the CB1 receptor open [84]. The concept that CB1 receptor activation could play a role in delayed protection is supported by data obtained in newborn rat pups exposed to acute asphyxia [85]. Pups treated with a low dose of Win 55212-2 (0.1 mg/kg) administered after the hypoxic period exhibited lower amounts of both early and delayed neuronal loss compared to controls; however, only the delayed neuronal loss was reversed by rimonabant. The delayed phase of cell death is primarily apoptotic. A mechanism for a long-term protective effect of CB1 receptor activation has been proposed. In particular, CB1 receptor activation results in induction of pro-survival factors, including brain derived growth factor (BDNF) [77] and basic fibroblast growth factor (bFGF) [86].

Several studies suggest further that endogenous cannabinoid signaling is recruited during excitotoxicity and that it exerts a protective effect. For example, seizures induced by kainic acid are far worse in mice with selective genetic deletion of the CB1 receptor in glutamate neurons of the hippocampus and in wild type mice treated with rimonabant [76,87]. On the other hand, genetic deletion of FAAH results in increased susceptibility to kainic acid-induced seizures [88]. Since these mice have elevated brain contents of AEA as well as the other NAEs [24], this finding seems to contradict the hypothesis that the ECS is protective. Since another study using pharmacological inhibition of FAAH demonstrated protection against kainic acid seizures [89], it is possible that life-long deletion of FAAH has induced other neurochemical changes. Interestingly, deletion of Gq/11 heterotrimeric G proteins results in both impaired eCB production and increased kainite-induced seizures [90], further support for an endogenous, neuroprotective ECS in this situation.

To summarize, there is convincing data to support the hypothesis that CB1 receptor activation protects against kainic acid-induced excitotoxicity and that this neuroprotection occurs “on demand”. In other words, the triggers of excitoxicity increase the activity of the ECS, likely through increased eCB synthesis. These ligands activate CB1 receptors at the synaptic terminals which could reduce further glutamate release and also induce the formation of pro-survival factors that oppose the effects of glutamate receptor activation. While these data are relevant to excitotoxicity in the brain in general, the lack of convincing data that endogenous cannabinoid signaling protects during ischemia/reperfusion calls into question whether these mechanisms contribute very much to the overall modulation of ischemic injury by changes in CB1 activation.

b. CB1 receptor activation produces hypothermia—Hypothermia has been shown to be neuroprotective in several models of ischemia [91,92]. The cannabinoids are well known to produce profound hypothermia [93,94] through a CB1-receptor dependent mechanism [95,96]. Therefore, the CB1 agonists could produce neuroprotection via their ability to reduce body temperature. Two in vivo studies support this mechanism. The cannabinoid, HU-210,

significantly reduced the infarct size in rats following permanent occlusion of the MCA [63]. HU 210 also produced a significant reduction in body temperature at 1 and 24 hours after its injection in rats with MCA occlusion; in fact, at 24 hours, the HU 210-treated rats had a nearly 6°C lower body temperature than rats treated with vehicle. Importantly, the protective effect of HU 210 to reduce infarct size was reversed partially by rimonabant and completely by warming of the rats to the temperatures of the controls. A second study of the protective effects of THC in mice with MCA occlusion also found that the neuroprotective effect of THC was reversed completely by warming [64]. These investigators also reported that rimonabant pretreatment attenuated both the neuroprotective and hypothermic effects of THC, supporting a role of the CB1 receptor in both effects. It is interesting to note that the protective effect of 2-AG in a model of traumatic injury was accompanied by a 1.6–1.9 °C drop in body temperature [44].

Whether CB1 agonist-induced hypothermia is completely responsible for their protective effects against ischemia is not clear. Most of the protocols in which ischemic injury is produced include methods for the monitoring and maintenance of body temperature during the surgical and anesthetic procedures. However, it is less likely that body temperature is maintained after the surgical intervention and the finding that HU 210 administration at the time of ischemia results in hypothermia 24 hours later suggests that hypothermia should not be discounted in these studies. Furthermore, in a study of the effects of transient global ischemia in gerbils, THC was found to reduce body temperature significantly even though a heating lamp was used in the protocol [69]. Therefore, it is very possible that brain hypothermia contributes significantly to the protective effects of the exogenously administered cannabinoid agonists against ischemic injury. Since rimonabant alone does not produce hyperthermia in normal rodents [97], it is not likely that this effect has endogenous tone, although it could during injury. The mechanism for this effect of the cannabinoid receptor is not clear, however, it could involve effects on nitric oxide synthase (NOS) activity since the hypothermic effect of THC is lost in nNOS null mice [98].

c. CB1 receptor activation reduces edema—Ischemia, reperfusion and other forms of brain injury are commonly accompanied by edema formation in the brain. Edema results in an expansion of brain volume resulting in an increase in intracranial pressure which can damage cells directly through compression and indirectly by impairment of cerebral perfusion. Therefore, a reduction in the degree of edema formation following ischemia is beneficial to the outcome of the injury. The CB1 agonists have been found to reduce edema following brain trauma [44] and in a model of excitotoxicity [82]. It is not clear whether the edema-lowering effect was a consequence of reducing damage through other mechanisms or was an independent, protective mechanism. There are several possible mechanisms by which CB1 agonists could reduce edema, including decreased systemic blood pressure [99] and increased glucocorticoid release in response to stress [100].

3. Evidence that reduced endocannabinoid signaling is protective against ischemia/reperfusion injury

As was discussed above, while there is good evidence that the AEA accumulates in the brain during ischemia, there is little evidence that blockade of the CB1 receptor during ischemia worsens the outcome of the insult. In fact, there are data from multiple laboratories that pharmacologic inhibition of the CB1 receptor at the time of the ischemia results in a reduction in the injury produced. These observations have lead to the hypothesis that increased ECS activation during ischemia contributes to the negative consequences of the injury (Table 1).

Berger, Schmid and colleagues reported that rats treated intravenously with 1 mg/kg of the CB1 receptor antagonist rimonabant (SR141716A) 30 min after permanent MCA occlusion

had infarct size that was reduced by 40% compared to vehicle controls at 5 hours [29]. Rimonabant has also been shown to reduce both the infarct size and neurological functional deficit induced by transient MCA occlusion in male rats while Win 55212-2 was without significant effect [30,31]. In one of these studies [30], a second, structurally unrelated CB1 receptor antagonist, LY 320135 [101], was also protective. Rimonabant is also protective in male gerbils exposed to transient, global ischemia [102]. The drug, administered 5 min after reperfusion and at low doses (less than 0.5 mg/kg), protected against the development of behavioral and EEG changes; in addition, animals given rimonabant exhibited nearly complete survival of cells in the CA1 region of the hippocampus 7 days after the ischemia. Interestingly, this effect of rimonabant was reversed by a low dose of capsazepine, a selective antagonist of transient receptor potential vanilloid 1 (TRPV1) receptors. TRPV1 receptors are activated by changes in temperature and pH as well as AEA and lipoxygenase products that accumulate during ischemia and can contribute to excitotoxicity [103]. Although the mechanism of this effect of rimonabant is unclear (see discussion section of [102] for a thorough discussion of the possibilities), this study provides strong support for a neuroprotective effect of the drug against ischemic/reperfusion injury.

Several other studies provide support for the hypothesis that reduced CB1 receptor signaling is protective against brain injury. Rimonabant was found to protect against NMDA-induced excitotoxicity in 6 day old rat pups while Win 55212-2 was without effect [104]. Interestingly, the protective effect was reversed by co-administration of Win 55212-2, supporting a role for the CB1 receptor in this effect of rimonabant. In another study, chronic treatment of rats with THC for 7 days at doses known to reduce CB1 receptor mRNA expression in the striatum but not the hippocampus [105] resulted in a significant reduction in ischemic injury in the striatum but not hippocampus [106]. The degree of protection in the striatum was remarkable, particularly since this brain region is notoriously insensitive to protective interventions. These authors suggest the intriguing possibility that the sensitivity of the striatum to ischemic damage is due to the high density of CB1 receptors found in this brain region and that chronic THC reduced the CB1 receptor density (through agonist-induced down-regulation), resulting in protection. Data demonstrating that short-term (2.5 min) exposure of gerbils to ischemia both protects the brain from damage in response to a longer ischemic period and decreases CB1 receptor density [53] also support the hypothesis that CB1 receptor activation can contribute to ischemic damage. Similarly, estrogen treatment of male rats prior to MCA occlusion results in decreased striatal CB1 receptor expression and decreased infarct in that brain region [31]. While these studies are correlative, they are intriguing and suggest that manipulation of CB1 receptor density as well as endogenous ligand concentrations could be therapeutic strategies to protect against ischemic injury. They suggest further that altered ECS signaling could contribute to the protective effects of other physiological manipulations.

The hypothesis that decreased CB1 receptor activation is neuroprotective is inconsistent with the finding that CB1 receptor null mice exhibit increased lethality following MCA occlusion [66] and that CB1 activation is protective in models of ischemia and other injury [61–64]. There are methodological differences between these studies and that of Nagayama and colleagues in which the CB1 agonist was shown to be neuroprotective [61], including different anesthetic agents (ketamine in the Berger study; halothane in the Muthian study and isoflurane in the Nagayama and Amantea studies); time at which damage was assessed (5 hours after occlusion in the Berger study; 24 hours later in the Nagayama, Amantea and Muthian studies) and the route of drug administration (intravenous in the Berger and Muthian studies and intraperitoneal in the Amantea and Nagayama studies). None of these methodological differences seems to explain the completely opposite results seen in these studies. It is possible that more subtle methodological differences have occurred and contribute to the results. For example, the degree to which the hypothermic effects of the cannabinoid agonists were counteracted with temperature regulation could contribute to differences in agonist effect. Another possibility is

that the drugs, both agonists and antagonists, could be acting through non-cannabinoid receptor targets to produce neuroprotection. In particular, the phenolic cannabinoids are antioxidant chemicals and can thereby act to reduce free radical damage [107,108]. The eCB, AEA, has many non-cannabinoid effects [109–111], including functioning as a precursor to ethanolamine, which inhibits serum withdrawal apoptosis in cell culture at surprisingly low concentrations [112]. Rimonabant also has off target effects; it can active MAPK signaling by non cannabinoid mechanisms [109] and inhibits the effects of AEA to activate TRPV1 receptors [110]. In light of the nonspecificity of the agents used, the fact that relatively high doses are sometimes required to either produce beneficial effects or, in the case of rimonabant, to antagonize the beneficial effects of agonists, it is quite possible that many of the effects ascribed to changes in CB1 receptor activity are, in fact, due to other mechanisms.

4. Mechanisms involved in the neuroprotective effects of CB1 receptor blockade

There are several possible mechanisms by which decreased CB1 receptor activation could result in reduced ischemic injury. First, CB1 receptors present on terminals of GABAergic interneurons in the hippocampus inhibit the release of GABA [113]. This mechanism of CB1 receptor activation will result in decreased inhibitory neurotransmission and could exacerbate excitotoxicity and CB1 receptor blockade would result in protection. Support for this hypothesis comes from data that increased GABAergic signaling reduces injury following ischemia and reperfusion in rats [114]. On the other hand, Marsicano and colleagues found that mice with a selective deletion of the CB1 receptor on glutamatergic neurons were more susceptible to kainic acid toxicity and were insensitive to rimonabant [76]. These data indicate that the CB1 receptors remaining in this preparation, which are on GABAergic interneurons, are not involved in the response to excitotoxicity. A recent paper by Navarrete and Araque has shown that astrocytes in the hippocampus express the CB1 receptor and that its activation by eCBs released from neurons results in increased glutamate release and subsequent activation of neuronal NMDA receptors [115]. Although the role of this signaling circuit has not been studied in ischemic brain, if operative, it could result in CB1 receptor-mediated excitotoxicity. In sum, there are signaling mechanisms that involve the CB1 receptor that theoretically could result in potentiation rather than reduction of excitotoxicity. However, the available evidence suggests that these processes are generally overwhelmed by the CB1 receptor mediated processes that result in decreased excitotoxicity.

A second possible mechanism is that rimonabant affects neurovascular coupling. As was discussed above, there is data in the literature to support the hypothesis that CB1 receptor activation protect neurons from excitotoxicity; however, excitotoxic damage is only one component of the complex changes that occur following ischemia and reperfusion. Therefore, it is possible that CB1 receptor activation exacerbates one or more of the other aspects of injury that occur or are present during ischemia but not during excitotoxicity per se. One hypothesis is that eCB activation of CB1 receptors of the cerebrovasculature worsens the damage produced by ischemia, possibly due to disruption of metabolic autoregulation or neurovascular coupling [2]. The neurovascular unit is the interface between the blood and the brain parenchyma and consists of microvascular endothelial cells, astrocytes and neurons. This unit is essential for proper brain function and determines the extent of neuronal damage following loss of blood flow. CB1 receptors are expressed by cerebral endothelial cells [17] and astrocytes [115] and there is evidence that AEA, through changes in endothelial NOS activity, alters capillary blood flow [116]. In addition, CB1 receptors are expressed by cerebral arterial smooth muscle cells [15] and the activation of these receptors results in vasodilation and increased blood flow [15,17,18]. While this might seem to be a beneficial effect of CB1 receptor activation during ischemia, it is likely detrimental as it will result in a loss of the ability of the circulation to autoregulate and to provide blood flow to regions in greatest need. Indeed, humans exposed to marijuana exhibit loss of cerebral autoregulation [20,117,118] possible through this

mechanism. In fact, there have been reports documenting the loss of cerebral autoregulation following stroke in humans [119,120] and in rat following MCA occlusion [121]. Therefore, these data suggest that activation of the CB1 receptor during times when the brain is dependent upon tight autoregulation to maintain perfusion homeostasis could be detrimental. This leads to the hypothesis that antagonism of the CB1 receptor during cerebral ischemia is protective because it allows for maintenance of proper autoregulation and/or neurovascular coupling. In support of this hypothesis, a recent study demonstrated that rimonabant treatment reduced the loss of cerebral blood flow in the area at risk in mice following transient MCA occlusion [50].

5. Evidence from in vitro models of nutrient loss

Studies have been carried out using various in vitro models of nutrient loss that provide insights into the role of changes in CB1 receptor activation to the metabolic consequences of these manipulations. The models in which excitotoxicity was induced were discussed above.

Cerebral cortical neurons in primary culture deprived of both glucose and oxygen for 8 hours undergo about 50% neuronal loss [61]. The presence of AEA and Win 55212-2 (10 nM) during hypoxia and glucose deprivation is protective, but THC increases cell loss. The effect of Win 55212-2 is not inhibited by rimonabant and is mimicked by Win 55212-3, a stereoisomer with much lower affinity for the CB1 receptor. Therefore, these studies demonstrate that the CB1 receptor agonist Win 55212-2 can protect neurons from nutrient deprivation at receptor-relevant concentrations but that the mechanism does not involve CB1 receptor activation. Phenolic cannabinoids, regardless of their affinity for the CB1 receptor, protect neurons in culture from oxidative injury likely due to chemical antioxidant properties [107,108,122]. However, Win 55212-2 does not appear to act via this mechanism [122].

Data suggesting that Win 55212-2 is anti-oxidant via a CB1 receptor-mediated mechanism is also available [123]. In this study, oxidative injury was produced in cortical neurons in primary culture through exposure to iron. This procedure results in a significant production of reactive oxygen radicals and 70% loss of neurons. Both Win 55212-2 (100 nM) and AEA (300 nM) were protective against cell death; rimonabant reversed the protection and Win 55212-2 was ineffective in neurons treated with pertussis toxin. The mechanism for this protection is not clear, but could involve decreased protein kinase A activity, a pathway known to result in the generation of reactive oxygen species [124].

6. Evidence that CB2 receptor activation is protective

Data from animal and human brain tissue have demonstrated that the presence of cells that express the CB2 cannabinoid receptor increases during many types of brain injury [125]. Furthermore, activation of CB2 receptors promotes cellular changes associated with decreased inflammation [14]. These data lead to the hypothesis that activation of the CB2 receptor by eCBs produced during ischemia could be neuroprotective. This hypothesis is supported by several studies, although this line of research is far less developed at this stage then the CB1 receptor-related research.

Among the first studies that raised the possibility that activation of CB2 cannabinoid receptors could protect against excitotoxic as well as inflammatory damage is an important study by Docagne and colleagues [126]. These investigators found that cannabinoid agonists with affinity for both CB1 and CB2 receptors prevented AMPA-induced cell injury both in vivo and in vitro. Importantly, pharmacological studies indicate that the beneficial effects of these agonists require activation of both CB1 and CB2 receptors. Studies carried out in mixed cortical cultures indicate that the CB1 receptors involved are neuronal while the CB2 receptors are on astrocytes. The results of this study are supported by those of Fernandez-Lopez and colleagues

using hypoxia-ischemia of newborn rats [127]. They found that Win 55212-2 was protective and that co-administration of either rimonabant or SR144528, an antagonist of CB2 receptors, reversed the effects of Win 55212-2. The authors conclude that the neuroprotective effects of an exogenous cannabinoid require both CB1 and CB2 receptor engagement for full effect. These studies are very interesting and could explain the incomplete blockade of the effects of Win 55212-2 by rimonabant in earlier studies (described above). However, these studies need to be confirmed with studies that do not rely on pharmacological methods.

The role of the CB2 receptor in focal ischemia/reperfusion has been examined in mice [128]. Treatment of mice before the ischemia or immediately after reperfusion with CB2 receptor agonists significantly reduced both infarct size and the motor disability induced by the insult. These authors also explored a possible mechanism of action for the CB2 receptor agonists. They used a closed cranial window preparation to visualize leukocyte rolling and adhesion in cerebral arterioles after the insult. These vascular changes are associated with increased inflammation and edema formation following ischemia and are an important contributor to the overall damage produced [129]. The CB2 agonists attenuated leukocyte rolling and adhesion in this model, which is an interesting and important potential mechanism of action of this class of drugs. In a second study using the mouse transient MCA occlusion model, this group found that the combination of a CB2 receptor agonist with a high dose of rimonabant (20 mg/kg) resulted in greater protection against the development of infarct than either drug alone [50]. Similarly, the combination therapy was also better than either drug alone in preventing the effects of occlusion on neurological deficits.

Therefore, it seems that ischemia can be added to the list of potential CNS disorders in which CB2 receptor activation holds promise as a therapeutic intervention. Since Win 55212-2 is a mixed CB receptor agonist, it is also possible that the neuroprotective effects ascribed to its ability to activate CB1 receptors could actually be due to its effects at the CB2 receptor. Finally, the studies of Zhang and colleagues add to the studies suggesting that CB1 receptor activation during ischemia is detrimental and that reducing this effect is also beneficial.

SUMMARY

As is pointed out in a recent series of opinion articles [130–132], there is currently a great deal of pessimism regarding neuroprotection as a treatment strategy for stroke; largely because none of the drugs that looked promising in animal studies have significant benefit in humans. However, there are good arguments for proceeding with research into neuroprotective strategies in spite of these disappointments. There is considerable evidence that ischemia evokes very similar biochemical changes in humans and animals; and many neuroprotective agents do work in animal models. Human stroke is an extremely complicated and heterogenous set of physiological circumstances that usually occur in people with other health problems; therefore, the hypothesis that a particular neuroprotective agents will work in all types of human stroke is likely naïve.

A picture is slowly emerging that the ECS is activated during ischemia and reperfusion of the brain. The concentrations of the ligands present are increased, as well as the concentrations of related lipids that do not activate the CB receptors. These changes are mediated by a combination of increased synthesis and decreased catabolism. In addition, there is evidence that the numbers of CB receptors change, likely through a combination of altered expression by resident cells and in-migration of circulating cells that express the CB2 receptor. Activation of the CB receptors leads to cellular changes that are extremely relevant to ischemic injury: they regulate glutamate release, nitric oxide synthesis, growth factor expression, cellular anti-oxidant activity, the release of inflammatory cytokines, and leukocyte adhesion to cerebral vessels (Fig. 1). Therefore, there is every reason to believe that the ECS contributes in some

way to the injury produced by ischemia. In light of these facts, the lack of a clear picture of the precise role of this system, is on one hand, puzzling. The answer at the moment seems to be that we do not completely appreciate the complexity of ECS signaling and that its manipulation using global pharmacological approaches has lead to incorrect conclusions. Future studies using more selective tools will certainly help to clarify this important and fascinating role of the ECS.

While it is possible that the ECS will be added to the long list of neuroprotective agents that show promise in animals and do not work in humans, there are a few reasons to be optimistic about this class of drugs. First, many of the other agents did not work because they do not cross the blood brain barrier. While the considerable lipophilicity of the cannabinoids poses its own set of problems, these drugs have no problems entering the brain. Second, the ECS is multifactorial and could “cover” multiple biochemical pathways in a single drug. Third, manipulations of the ECS has been shown to be beneficial in several preclinical models. Only time and further research will answer the most important question, are the cannabinoids of therapeutic benefit in humans suffering from stroke?

Acknowledgements

The author was supported during the writing of this review by NIH grant DA09155.

References

1. Lipton P. Ischemic cell death in brain neurons. Physiol Rev 1999;79:1431–568. [PubMed: 10508238]
2. Mehta SL, Manhas N, Raghubir R. Molecular targets in cerebral ischemia for developing novel therapeutics. Brain Res Rev 2007;54:34–66. [PubMed: 17222914]
3. Albers GW, Goldberg MP, Choi DW. N-methyl-D-aspartate antagonists: ready for clinical trial in brain ischemia? Ann Neurol 1989;25:398–403. [PubMed: 2565699]
4. Natarajan V, Reddy PV, Schmid PC, Schmid HHO. On the biosynthesis and metabolism of n-acylethanolamine phospholipids in infarcted dog heart. Biochim Biophys Acta 1981;664:445–8. [PubMed: 7248333]
5. Schmid PC, Krebsbach RJ, Perry SR, Dettmer TM, Maasson JL, Schmid HH. Occurrence and postmortem generation of anandamide and other long-chain N-acylethanolamines in mammalian brain. FEBS Lett 1995;375:143–7. [PubMed: 7498464]
6. Kunos G. Understanding metabolic homeostasis and imbalance: what is the role of the endocannabinoid system? Am J Med 2007;120:S18–24. [PubMed: 17720356]
7. Gorzalka BB, Hill MN, Hillard CJ. Regulation of endocannabinoid signaling by stress: Implications for stress-related affective disorders. Neurosci Biobehav Rev. 2008
8. Caulfield MP, Brown DA. Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. Br J Pharmacol 1992;106:231–2. [PubMed: 1327374]
9. Mackie K, Hille B. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. Proc Natl Acad Sci 1992;89:3825–9. [PubMed: 1315042]
10. Nogueron MI, Porgilsson B, Schneider WE, Stucky CL, Hillard CJ. Cannabinoid receptor agonists inhibit depolarization-induced calcium influx in cerebellar granule neurons. J Neurochem 2001;79:371–81. [PubMed: 11677265]
11. Shen M, Piser TM, Seybold VS, Thayer SA. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci 1996;16:4322–34. [PubMed: 8699243]
12. Bouaboula M, Rinaldi M, Carayon P, Carillon C, Delpech B, Shire D, et al. Cannabinoid-receptor expression in human leukocytes. Eur J Biochem 1993;214:173–80. [PubMed: 8508790]
13. Maresz K, Carrier EJ, Ponomarev ED, Hillard CJ, Dittel BN. Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. J Neurochem 2005;95:437–45. [PubMed: 16086683]

14. Carrier EJ, Patel S, Hillard CJ. Endocannabinoids in neuroimmunology and stress. Curr Drug Targets CNS Neurol Disord 2005;4:657–65. [PubMed: 16375683]
15. Gebremedhin D, Lange AR, Campbell WB, Hillard CJ, Harder DR. Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca^{2+} channel current. Am J Physiol 1999;276:H2085–H93. [PubMed: 10362691]
16. Chen Y, McCarron RM, Ohara Y, Bembry J, Azzam N, Lenz FA, et al. Human brain capillary endothelium: 2-arachidonoglycerol (endocannabinoid) interacts with endothelin-1. Circ Res 2000;87:323–7. [PubMed: 10948067]
17. Wagner JA, Jarai Z, Batkai S, Kunos G. Hemodynamic effects of cannabinoids: coronary and cerebral vasodilation mediated by cannabinoid CB(1) receptors. Eur J Pharmacol 2001;423:203–10. [PubMed: 11448486]
18. Rademacher DJ, Patel S, Ho WS, Savoie AM, Rusch NJ, Gauthier KM, et al. U-46619 but not serotonin increases endocannabinoid content in middle cerebral artery: evidence for functional relevance. Am J Physiol Heart Circ Physiol 2005;288:H2694–701. [PubMed: 15695564]
19. Hillard CJ, Ho WS, Thompson J, Gauthier KM, Wheelock CE, Huang H, et al. Inhibition of 2-arachidonoylglycerol catabolism modulates vasoconstriction of rat middle cerebral artery by the thromboxane mimetic, U-46619. Br J Pharmacol. 2007
20. Mathew RJ, Wilson WH, Davis R. Postural syncope after marijuana: a transcranial Doppler study of the hemodynamics. Pharmacol Biochem Behav 2003;75:309–18. [PubMed: 12873621]
21. Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev 2003;83:1017–66. [PubMed: 12843414]
22. Felder CC, Nielsen A, Briley EM, Palkovits M, Priller J, Axelrod J, et al. Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. FEBS Lett 1996;393:231–5. [PubMed: 8814296]
23. Kempe K, Hsu FF, Bohrer A, Turk J. Isotope dilution mass spectrometric measurements indicate that arachidonylethanolamide, the proposed endogenous ligand of the cannabinoid receptor, accumulates in rat brain tissue post mortem but is contained at low levels in or is absent from fresh tissue. J Biol Chem 1996;271:17287–95. [PubMed: 8663381]
24. Patel S, Carrier EJ, Ho WS, Rademacher DJ, Cunningham S, Reddy DS, et al. The postmortal accumulation of brain N-arachidonylethanolamine (anandamide) is dependent upon fatty acid amide hydrolase activity. J Lipid Res 2005;46:342–9. [PubMed: 15576840]
25. Moesgaard B, Jaroszewski JW, Hansen HS. Accumulation of N-acyl-ethanolamine phospholipids in rat brains during post-decapitative ischemia: a (31)P NMR study. J Lipid Res 1999;40:515–21. [PubMed: 10064740]
26. Natarajan V, Schmid P, Schmid H. N-Acylethanolamine phospholipid metabolism in normal and ischemic rat brain. Biochim Biophys Acta 1986;878:32–41. [PubMed: 3730413]
27. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 1996;384:83–7. [PubMed: 8900284]
28. Sugiura T, Yoshinaga N, Waku K. Rapid generation of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, in rat brain after decapitation. Neurosci Lett 2001;297:175–8. [PubMed: 11137756]
29. Berger C, Schmid PC, Schabitz WR, Wolf M, Schwab S, Schmid HHO. Massive accumulation of N-acylethanolamines after stroke. Cell signalling in acute cerebral ischemia? J Neurochem 2004;88:1159–67. [PubMed: 15009671]
30. Muthian S, Rademacher DJ, Roelke CT, Gross GJ, Hillard CJ. Anandamide content is increased and CB1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia. Neuroscience 2004;129:743–50. [PubMed: 15541895]
31. Amantea D, Spagnuolo P, Bari M, Fezza F, Mazzei C, Tassorelli C, et al. Modulation of the endocannabinoid system by focal brain ischemia in the rat is involved in neuroprotection afforded by 17beta-estradiol. Febs J 2007;274:4464–775. [PubMed: 17666109]
32. Degn M, Lambertsen KL, Petersen G, Meldgaard M, Artmann A, Clausen BH, et al. Changes in brain levels of N-acylethanolamines and 2-arachidonoylglycerol in focal cerebral ischemia in mice. J Neurochem 2007;103:1907–16. [PubMed: 17868306]

33. Hillard CJ. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. Prostaglandins Other Lipid Mediat 2000;61:3–18. [PubMed: 10785538]
34. Astarita G, Ahmed F, Piomelli D. Identification of biosynthetic precursors for the endocannabinoid anandamide in the rat brain. J Lipid Res 2008;49:48–57. [PubMed: 17957091]
35. Tsuboi K, Zhao LY, Okamoto Y, Araki N, Ueno M, Sakamoto H, et al. Predominant expression of lysosomal N-acylethanolamine-hydrolyzing acid amidase in macrophages revealed by immunochemical studies. Biochim Biophys Acta 2007;1771:623–32. [PubMed: 17462942]
36. Xiao AZ, Zhao YG, Duan EK. Expression and regulation of the fatty acid amide hydrolase gene in the rat uterus during the estrous cycle and peri-implantation period. Mol Hum Reprod 2002;8:651–8. [PubMed: 12087080]
37. Waleh NS, Cravatt BF, Apte-Deshpande A, Terao A, Kilduff TS. Transcriptional regulation of the mouse fatty acid amide hydrolase gene. Gene 2002;291:203–10. [PubMed: 12095693]
38. Hill MN, Karacabeyli ES, Gorzalka BB. Estrogen recruits the endocannabinoid system to modulate emotionality. Psychoneuroendocrinology 2007;32:350–7. [PubMed: 17391861]
39. Alkayed NJ, Harukuni I, Kimes AS, London ED, Traystman RJ, Hurn PD. Gender-linked brain injury in experimental stroke. Stroke 1998;29:159–65. [PubMed: 9445346]
40. Hansen HS, Moesgaard B, Hansen HH, Schousboe A, Petersen G. Formation of N-acyl-phosphatidylethanolamine and N-acylethanolamine (including anandamide) during glutamate-induced neurotoxicity. Lipids 1999;34(Suppl):S327–30. [PubMed: 10419193]
41. Franklin A, Parmentier-Batteur S, Walter L, Greenberg DA, Stella N. Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. J Neurosci 2003;23:7767–75. [PubMed: 12944505]
42. Schabitz WR, Giuffrida A, Berger C, Aschoff A, Schwaninger M, Schwab S, et al. Release of fatty acid amides in a patient with hemispheric stroke: a microdialysis study. Stroke 2002;33:2112–4. [PubMed: 12154273]
43. Sugiura T, Yoshinaga N, Kondo S, Waku K, Ishima Y. Generation of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, in picrotoxinin-administered rat brain. Biochem Biophys Res Commun 2000;271:654–8. [PubMed: 10814517]
44. Panikashvili D, Simeonidou C, Ben-Shabat S, Hanus L, Breuer A, Mechoulam R, et al. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature 2001;413:527–31. [PubMed: 11586361]
45. Witting A, Weydt P, Hong S, Kliot M, Moller T, Stella N. Endocannabinoids accumulate in spinal cord of SOD1 G93A transgenic mice. J Neurochem 2004;89:1555–7. [PubMed: 15189359]
46. Hansen HH, Ikonomidou C, Bittigau P, Hansen SH, Hansen HS. Accumulation of the anandamide precursor and other N-acylethanolamine phospholipids in infant rat models of in vivo necrotic and apoptotic neuronal death. J Neurochem 2001;76:39–46. [PubMed: 11145976]
47. Lees GJ, Lehmann A, Sandberg M, Hamberger A. The neurotoxicity of ouabain, a sodium-potassium ATPase inhibitor, in the rat hippocampus. Neurosci Lett 1990;120:159–62. [PubMed: 1705675]
48. van der Stelt M, Veldhuis WB, van Haaften GW, Fezza F, Bisogno T, Bar PR, et al. Exogenous anandamide protects rat brain against acute neuronal injury in vivo. J Neurosci 2001;21:8765–71. [PubMed: 11698588]
49. Jin KL, Mao XO, Goldsmith PC, Greenberg DA. CB1 cannabinoid receptor induction in experimental stroke. Ann Neurol 2000;48:257–61. [PubMed: 10939579]
50. Zhang M, Martin BR, Adler MW, Razdan RK, Ganea D, Tuma RF. Modulation of the balance between cannabinoid CB(1) and CB(2) receptor activation during cerebral ischemic/reperfusion injury. Neuroscience 2008;152:753–60. [PubMed: 18304750]
51. Sommer C, Schomacher M, Berger C, Kuhnert K, Muller HD, Schwab S, et al. Neuroprotective cannabinoid receptor antagonist SR141716A prevents downregulation of excitotoxic NMDA receptors in the ischemic penumbra. Acta Neuropathol (Berl) 2006;112:277–86. [PubMed: 16871404]
52. Hansen HH, Schmid PC, Bittigau P, Lastres-Becker I, Berrendero F, Manzanares J, et al. Anandamide, but not 2-arachidonoylglycerol, accumulates during in vivo neurodegeneration. J Neurochem 2001;78:1415–27. [PubMed: 11579150]

53. Schomacher M, Muller HD, Sommer C. Short-term ischemia usually used for ischemic preconditioning down-regulates central cannabinoid receptors in the gerbil hippocampus. Acta Neuropathol (Berl) 2006;111:8–14. [PubMed: 16328514]
54. Lee JM, Zipfel GJ, Choi DW. The changing landscape of ischaemic brain injury mechanisms. Nature 1999;399:A7–14. [PubMed: 10392575]
55. Hayakawa K, Mishima K, Nozako M, Hazekawa M, Aoyama Y, Ogata A, et al. High-cholesterol feeding aggravates cerebral infarction via decreasing the CB1 receptor. Neurosci Lett 2007;414:183–7. [PubMed: 17208374]
56. Nunez E, Benito C, Pazos MR, Barbachano A, Fajardo O, Gonzalez S, et al. Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. Synapse 2004;53:208–13. [PubMed: 15266552]
57. Benito C, Kim WK, Chavarria I, Hillard CJ, Mackie K, Tolon RM, et al. A glial endogenous cannabinoid system is upregulated in the brains of macaques with simian immunodeficiency virus-induced encephalitis. J Neurosci 2005;25:2530–6. [PubMed: 15758162]
58. Maresz K, Pryce G, Ponomarev ED, Marsicano G, Croxford JL, Shriver LP, et al. Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. Nat Med 2007;13:492–7. [PubMed: 17401376]
59. Ashton JC, Rahman RM, Nair SM, Sutherland BA, Glass M, Appleton I. Cerebral hypoxia-ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB2 receptor in the brain. Neurosci Lett 2007;412:114–7. [PubMed: 17123706]
60. Nunez E, Benito C, Tolon RM, Hillard CJ, Griffin WS, Romero J. Glial expression of cannabinoid CB(2) receptors and fatty acid amide hydrolase are beta amyloid-linked events in Down's syndrome. Neuroscience 2008;151:104–10. [PubMed: 18068305]
61. Nagayama T, Sinor AD, Simon RP, Chen J, Graham SH, Jin K, et al. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. J Neurosci 1999;19:2987–95. [PubMed: 10191316]
62. Mauler F, Mittendorf J, Horvath E, De Vry J. Characterization of the diarylether sulfonylester (-)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38–7271) as a potent cannabinoid receptor agonist with neuroprotective properties. J Pharmacol Exp Ther 2002;302:359–68. [PubMed: 12065738]
63. Leker RR, Gai N, Mechoulam R, Ovadia H. Drug-induced hypothermia reduces ischemic damage: effects of the cannabinoid HU-210. Stroke 2003;34:2000–6. [PubMed: 12829867]
64. Hayakawa K, Mishima K, Nozako M, Hazekawa M, Ogata A, Fujioka M, et al. Delta(9)-tetrahydrocannabinol (Delta(9)-THC) prevents cerebral infarction via hypothalamic-independent hypothermia. Life Sci 2007;80:1466–71. [PubMed: 17289082]
65. Braida D, Pozzi M, Sala M. CP 55,940 protects against ischemia-induced electroencephalographic flattening and hyperlocomotion in Mongolian gerbils. Neurosci Lett 2000;296:69–72. [PubMed: 11108983]
66. Parmentier-Batteur S, Jin K, Mao XO, Xie L, Greenberg DA. Increased severity of stroke in CB1 cannabinoid receptor knock-out mice. J Neurosci 2002;22:9771–5. [PubMed: 12427832]
67. Schmid P, Zuzarte-Augustin M, Schmid H. Properties of rat liver N-acylethanolamine amidohydrolase. J Biol Chem 1985;260:14145–9. [PubMed: 4055775]
68. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 1997;277:1094–7. [PubMed: 9262477]
69. Zani A, Braida D, Capurro V, Sala M. Delta9-tetrahydrocannabinol (THC) and AM 404 protect against cerebral ischaemia in gerbils through a mechanism involving cannabinoid and opioid receptors. Br J Pharmacol 2007;152:1301–11. [PubMed: 17965746]
70. Shen M, Thayer SA. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. Molec Pharmacol 1998;54:459–62. [PubMed: 9730904]
71. Gilbert GL, Kim HJ, Waataja JJ, Thayer SA. Delta9-tetrahydrocannabinol protects hippocampal neurons from excitotoxicity. Brain Res 2007;1128:61–9. [PubMed: 17140550]
72. Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neurosci 1998;83:393–411.

73. Deadwyler SA, Hampson RE, Bennett BA, Edwards TA, Mu J, Pacheco MA, et al. Cannabinoids modulate potassium current in cultured hippocampal neurons. Receptors Channels 1993;1:121–34. [PubMed: 8081716]
74. Hillard CJ, Muthian S, Kearn CS. Effects of CB(1) cannabinoid receptor activation on cerebellar granule cell nitric oxide synthase activity. FEBS Lett 1999;459:277–81. [PubMed: 10518035]
75. Abood ME, Rizvi G, Sallapudi N, McAllister SD. Activation of the CB1 cannabinoid receptor protects cultured mouse spinal neurons against excitotoxicity. Neurosci Lett 2001;309:197–201. [PubMed: 11514075]
76. Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 2003;302:84–8. [PubMed: 14526074]
77. Khaspekov LG, Brenz Verca MS, Frumkina LE, Hermann H, Marsicano G, Lutz B. Involvement of brain-derived neurotrophic factor in cannabinoid receptor-dependent protection against excitotoxicity. Eur J Neurosci 2004;19:1691–8. [PubMed: 15078543]
78. Guan XL, He X, Ong WY, Yeo WK, Shui G, Wenk MR. Non-targeted profiling of lipids during kainate-induced neuronal injury. Faseb J 2006;20:1152–61. [PubMed: 16770014]
79. Andersson M, Jacobsson SO, Jonsson KO, Tiger G, Fowler CJ. Neurotoxicity of glutamate in chick telencephalon neurons: reduction of toxicity by preincubation with carbachol, but not by the endogenous fatty acid amides anandamide and palmitoylethanolamide. Faseb J 2000;14:1432–8. [PubMed: 10877836]
80. Hampson AJ, Grimaldi M. Cannabinoid receptor activation and elevated cyclic AMP reduce glutamate neurotoxicity. Eur J Neurosci 2001;13:1529–36. [PubMed: 11328347]
81. Youssef FF, Hormuzdi SG, Irving AJ, Frenguelli BG. Cannabinoid modulation of neuronal function after oxygen/glucose deprivation in area CA1 of the rat hippocampus. Neuropharmacology 2007;52:1327–35. [PubMed: 17382973]
82. van der Stelt M, Veldhuis WB, Bar PR, Veldink GA, Vliegenthart JF, Nicolay K. Neuroprotection by Delta9-tetrahydrocannabinol, the main active compound in marijuana, against ouabain-induced in vivo excitotoxicity. J Neurosci 2001;21:6475–9. [PubMed: 11517236]
83. Veldhuis WB, van der Stelt M, Wadman MW, van Zadelhoff G, Maccarrone M, Fezza F, et al. Neuroprotection by the endogenous cannabinoid anandamide and arvanil against in vivo excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases. J Neurosci 2003;23:4127–33. [PubMed: 12764100]
84. Shouman B, Fontaine RH, Baud O, Schwendimann L, Keller M, Spedding M, et al. Endocannabinoids potently protect the newborn brain against AMPA-kainate receptor-mediated excitotoxic damage. Br J Pharmacol 2006;148:442–51. [PubMed: 16682966]
85. Martinez-Orgado J, Fernandez-Frutos B, Gonzalez R, Romero E, Uriguen L, Romero J, et al. Neuroprotection by the cannabinoid agonist WIN-55212 in an in vivo newborn rat model of acute severe asphyxia. Brain Res Mol Brain Res 2003;114:132–9. [PubMed: 12829323]
86. Aguado T, Romero E, Monory K, Palazuelos J, Sendtner M, Marsicano G, et al. The CB1 cannabinoid receptor mediates excitotoxicity-induced neural progenitor proliferation and neurogenesis. J Biol Chem 2007;282:23892–8. [PubMed: 17556369]
87. Monory K, Massa F, Egertova M, Eder M, Blaudzun H, Westenbroek R, et al. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron 2006;51:455–66. [PubMed: 16908411]
88. Clement AB, Hawkins EG, Lichtman AH, Cravatt BF. Increased seizure susceptibility and proconvulsant activity of anandamide in mice lacking fatty acid amide hydrolase. J Neurosci 2003;23:3916–23. [PubMed: 12736361]
89. Karanian DA, Karim SL, Wood JT, Williams JS, Lin S, Makriyannis A, et al. Endocannabinoid Enhancement Protects against Kainic Acid-Induced Seizures and Associated Brain Damage. J Pharmacol Exp Ther 2007;322:1059–66. [PubMed: 17545313]
90. Wettschureck N, van der Stelt M, Tsubokawa H, Krestel H, Moers A, Petrosino S, et al. Forebrain-specific inactivation of Gq/G11 family G proteins results in age-dependent epilepsy and impaired endocannabinoid formation. Mol Cell Biol 2006;26:5888–94. [PubMed: 16847339]

91. Krieger DW, Yenari MA. Therapeutic hypothermia for acute ischemic stroke: what do laboratory studies teach us? Stroke 2004;35:1482–9. [PubMed: 15073396]

92. Hoesch RE, Geocadin RG. Therapeutic hypothermia for global and focal ischemic brain injury--a cool way to improve neurologic outcomes. Neurologist 2007;13:331–42. [PubMed: 18090711]

93. Haavik CO, Hardman HF. Hypothermic action of delta 9-tetrahydrocannabinol, 11-hydroxy-delta 9-tetrahydrocannabinol and 11-hydroxy-delta 8-tetrahydrocannabinol in mice. Life Sci 1973;13:1771–8. [PubMed: 4777336]

94. Haavik CO, Hardman HF. Evaluation of the hypothermic action of tetrahydrocannabinols in mice and squirrel monkeys. J Pharmacol Exp Ther 1973;187:568–74. [PubMed: 4203720]

95. Compton DR, Gold LH, Ward SJ, Balster RL, Martin BR. Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from delta 9-tetrahydrocannabinol. J Pharmacol Exp Ther 1992;263:1118–26. [PubMed: 1335057]

96. Hillard CJ, Manna S, Greenberg MJ, DiCamelli R, Ross RA, Stevenson LA, et al. Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). J Pharmacol Exp Ther 1999;289:1427–33. [PubMed: 10336536]

97. McMahon LR, Koek W. Differences in the relative potency of SR 141716A and AM 251 as antagonists of various in vivo effects of cannabinoid agonists in C57BL/6J mice. Eur J Pharmacol 2007;569:70–6. [PubMed: 17553486]

98. Azad SC, Marsicano G, Eberlein I, Putzke J, Zieglgansberger W, Spanagel R, et al. Differential role of the nitric oxide pathway on delta(9)-THC-induced central nervous system effects in the mouse. Eur J Neurosci 2001;13:561–8. [PubMed: 11168564]

99. Mendizabal VE, Adler-Graschinsky E. Cannabinoids as therapeutic agents in cardiovascular disease: a tale of passions and illusions. Br J Pharmacol 2007;151:427–40. [PubMed: 17450170]

100. Patel S, Roelke CT, Rademacher DJ, Cullinan WE, Hillard CJ. Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis. Endocrinology 2004;145:5431–8. [PubMed: 15331569]

101. Felder CC, Joyce KE, Briley EM, Glass M, Mackie KP, Fahey KJ, et al. LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. J Pharmacol Exp Ther 1998;284:291–7. [PubMed: 9435190]

102. Pegorini S, Zani A, Braida D, Guerini-Rocco C, Sala M. Vanilloid VR1 receptor is involved in rimonabant-induced neuroprotection. Br J Pharmacol 2006;147:552–9. [PubMed: 16444289]

103. Marinelli S, Vaughan CW, Christie MJ, Connor M. Capsaicin activation of glutamatergic synaptic transmission in the rat locus coeruleus in vitro. J Physiol 2002;543:531–40. [PubMed: 12205187]

104. Hansen HH, Azcoitia I, Pons S, Romero J, Garcia-Segura LM, Ramos JA, et al. Blockade of cannabinoid CB(1) receptor function protects against in vivo disseminating brain damage following NMDA-induced excitotoxicity. J Neurochem 2002;82:154–8. [PubMed: 12091476]

105. Zhuang S, Kittler J, Grigorenko EV, Kirby MT, Sim LJ, Hampson RE, et al. Effects of long-term exposure to delta9-THC on expression of cannabinoid receptor (CB1) mRNA in different rat brain regions. Brain Res Mol Brain Res 1998;62:141–9. [PubMed: 9813289]

106. Louw DF, Yang FW, Sutherland GR. The effect of delta-9-tetrahydrocannabinol on forebrain ischemia in rat. Brain Res 2000;857:337–42. [PubMed: 10700588]

107. Hampson AJ, Grimaldi M, Axelrod J, Wink D. Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci U S A 1998;95:8268–73. [PubMed: 9653176]

108. Marsicano G, Moosmann B, Hermann H, Lutz B, Behl C. Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. J Neurochem 2002;80:448–56. [PubMed: 11905991]

109. Berdyshev EV, Schmid PC, Krebsbach RJ, Hillard CJ, Huang C, Chen N, et al. Cannabinoid-receptor-independent cell signalling by N-acylethanolamines. Biochem J 2001;360:67–75. [PubMed: 11695993]

110. De Petrocellis L, Bisogno T, Maccarrone M, Davis JB, Finazzi-Agro A, Di Marzo V. The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. J Biol Chem 2001;26:26.

111. Venance L, Piomelli D, Glowinski J, Giaume C. Inhibition by anandamide of gap junctions and intercellular calcium signalling in striatal astrocytes. Nature 1995;376:590–4. [PubMed: 7637807]

112. Matas D, Juknat A, Pietr M, Klin Y, Vogel Z. Anandamide protects from low serum-induced apoptosis via its degradation to ethanolamine. J Biol Chem 2007;282:7885–92. [PubMed: 17227767]

113. Varma N, Carlson GC, Ledent C, Alger BE. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. J Neurosci 2001;21:RC188. [PubMed: 11734603]

114. Xu J, Li C, Yin XH, Zhang GY. Additive neuroprotection of GABA A and GABA B receptor agonists in cerebral ischemic injury via PI-3K/Akt pathway inhibiting the ASK1-JNK cascade. Neuropharmacology 2008;54:1029–40. [PubMed: 18410948]

115. Navarrete M, Araque A. Endocannabinoids mediate neuron-astrocyte communication. Neuron 2008;57:883–93. [PubMed: 18367089]

116. Prevot V, Rialas CM, Croix D, Salzet M, Dupouy JP, Poulain P, et al. Morphine and anandamide coupling to nitric oxide stimulates GnRH and CRF release from rat median eminence: neurovascular regulation. Brain Res 1998;790:236–44. [PubMed: 9593913]

117. Mathew, R.; Wilson, W. The effects of marijuana on cerebral blood flow and metabolism. In: Bartke, A.; Murphy, L., editors. Marijuana/Cannabinoids: Neurobiology and Neurophysiology. CRC Press; Boca Raton, FL: 1992. p. 337-86.

118. Mathew R, Wilson W, Humphreys D, Lowe J, Wiethe K. Changes in middle cerebral artery velocity after marijuana. Biol Psychiat 1992;32:164–9. [PubMed: 1420630]

119. Eames PJ, Blake MJ, Dawson SL, Panerai RB, Potter JF. Dynamic cerebral autoregulation and beat to beat blood pressure control are impaired in acute ischaemic stroke. J Neurol Neurosurg Psychiatry 2002;72:467–72. [PubMed: 11909905]

120. Novak V, Chowdhary A, Farrar B, Nagaraja H, Braun J, Kanard R, et al. Altered cerebral vasoregulation in hypertension and stroke. Neurology 2003;60:1657–63. [PubMed: 12771258]

121. MacGregor DG, Carswell HVO, Graham DI, McCulloch J, Macrae IM. Impaired cerebral autoregulation 24h after induction of transient unilateral focal ischaemia in the rat. Eur J Neurosci 2000;12:58–66. [PubMed: 10651860]

122. Chen Y, Buck J. Cannabinoids protect cells from oxidative cell death: a receptor-independent mechanism. J Biochem Biophys Methods 2000;43:261–72. [PubMed: 10869681]

123. Kim SH, Won SJ, Mao XO, Jin K, Greenberg DA. Involvement of protein kinase a in cannabinoid receptor-mediated protection from oxidative neuronal injury. J Pharmacol Exp Ther 2005;313:88–94. [PubMed: 15626718]

124. Boissel JP, Bros M, Schrock A, Godtel-Armbrust U, Forstermann U. Cyclic AMP-mediated upregulation of the expression of neuronal NO synthase in human A673 neuroepithelioma cells results in a decrease in the level of bioactive NO production: analysis of the signaling mechanisms that are involved. Biochemistry 2004;43:7197–206. [PubMed: 15170357]

125. Arevalo-Martin A, Garcia-Ovejero D, Gomez O, Rubio-Araiz A, Navarro-Galve B, Guaza C, et al. CB(2) cannabinoid receptors as an emerging target for demyelinating diseases: from neuroimmune interactions to cell replacement strategies. Br J Pharmacol 2008;153:216–25. [PubMed: 17891163]

126. Docagne F, Muneton V, Clemente D, Ali C, Loria F, Correa F, et al. Excitotoxicity in a chronic model of multiple sclerosis: Neuroprotective effects of cannabinoids through CB1 and CB2 receptor activation. Mol Cell Neurosci. 2007

127. Fernandez-Lopez D, Pazos MR, Tolon RM, Moro MA, Romero J, Lizasoain I, et al. The Cannabinoid Agonist Win55212 Reduces Brain Damage in an In Vivo Model of Hypoxic-Ischemic Encephalopathy in Newborn Rats. Pediatr Res 2007;63:255–60. [PubMed: 17622949]

128. Zhang M, Martin BR, Adler MW, Razdan RK, Jallo JI, Tuma RF. Cannabinoid CB(2) receptor activation decreases cerebral infarction in a mouse focal ischemia/reperfusion model. J Cereb Blood Flow Metab 2007;27:1387–96. [PubMed: 17245417]

129. Sughrue ME, Mehra A, Connolly ES Jr, D'Ambrosio AL. Anti-adhesion molecule strategies as potential neuroprotective agents in cerebral ischemia: a critical review of the literature. Inflamm Res 2004;53:497–508. [PubMed: 15597143]

130. Donnan GA, Davis SM. Neuroprotection: still achievable in humans. Stroke 2008;39:525. [PubMed: 18202308]

131. Hussain MS, Shuaib A. Research into neuroprotection must continue … But with a different approach. Stroke 2008;39:521–2. [PubMed: 18202310]

132. Rother J. Neuroprotection does not work! Stroke 2008;39:523–4. [PubMed: 18202309]

Figure 1 A.

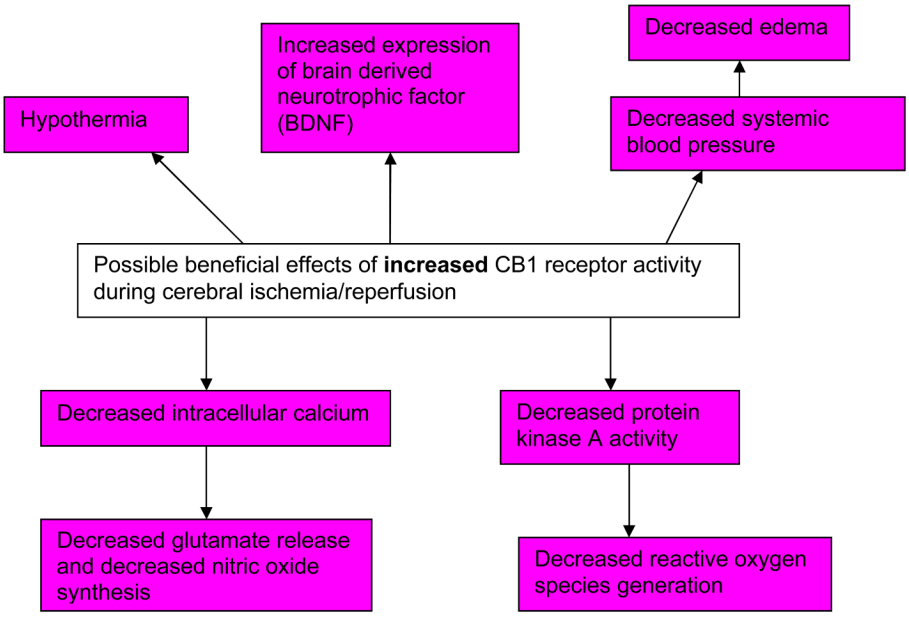

Figure 1.B.

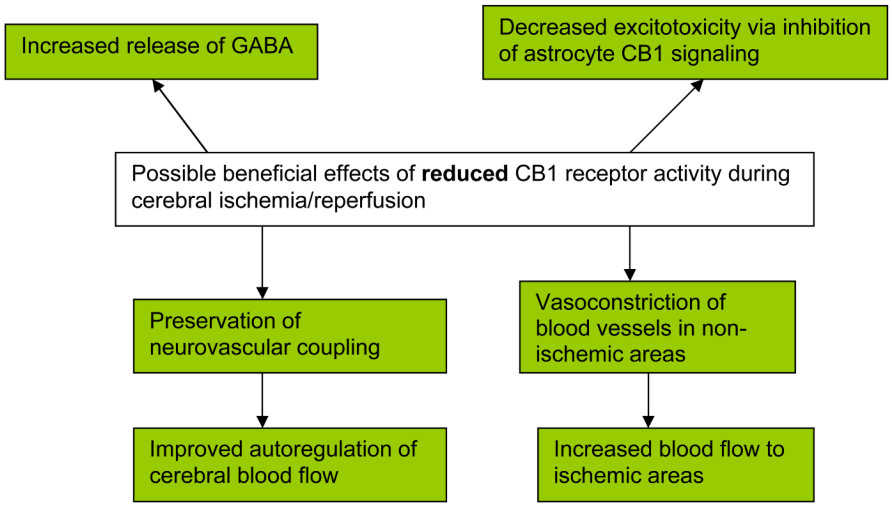

Figure 1.C.

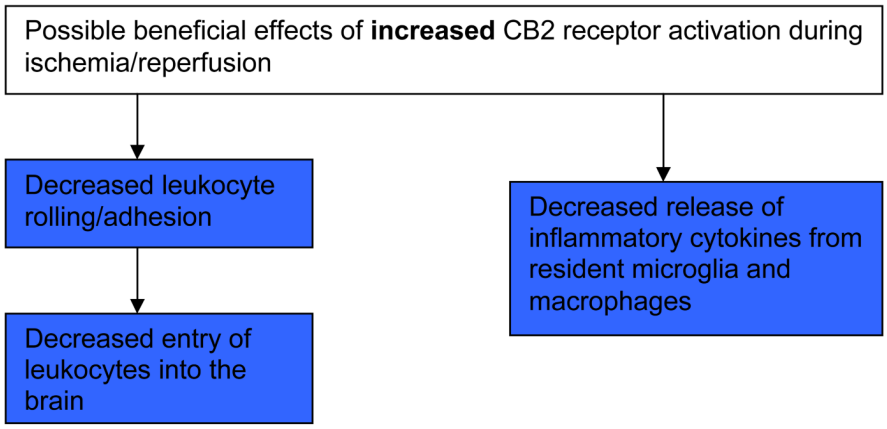

Figure 1.
Summary of the possible mechanisms involved in the neuroprotective effects of cannabinoid receptor activation or inhibition. See text for details and supporting citations.

Table 1

Summary of studies demonstrating neuroprotective effects of cannabinoid receptor ligands in in vivo ischemia models

Species	Ischemia model	Drug class	Results	References
Rat	Transient, global	CB agonist	Protection; reversed by CB1 antagonist	61
Rat	Permanent, focal	CB agonist	Protection	61,62,63
Mouse	Transient, focal	CB agonist	Protection; reversed by warming	64
Gerbil	Transient, global	CB agonist	Protection; reversed by CB1 antagonist	65
Rat (neonatal)	Hypoxia and ischemia	CB agonist	Protection, reversed by both CB1 and CB2 antagonists	127
Rat	Transient, focal	CB1 agonist	No effect	30,31
Mouse	Permanent, focal	FAAH inhibitor	Protection; CB1 role undetermined	32
Gerbil	Transient, global	FAAH/AEA uptake inhibitor	Protection; not CB1 dependent	69
Mouse	Permanent, focal	Genetic deletion of CB1 receptor	Increased damage in knock out	66
Rat	Permanent, focal	CB1 antagonist	Protection	29
Rat	Transient, focal	CB1 antagonist	Protection	30,31
Gerbil	Transient, global	CB1 antagonist	Protection	102
Mouse	Transient, focal	CB2 agonist	Protection; CB2 receptor dependent	128
Mouse	Transient, focal	CB2 agonist + CB1 antagonist	Additive protection	50
Rat	Transient, global	Chronic THC treatment	Protection in some brain regions	106

See text for more details regarding these studies.