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Endocannabinoid Regulation of Matrix Metalloproteinases: Implications in Ischemic Stroke

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Abstract

Stroke is a major cause of morbidity and mortality and follows heart disease and cancer as the third leading cause of death in Western societies [1]. Despite many advances in stroke research and pharmacotherapy, clinical treatment of this debilitating disorder is still inadequate. Recent findings from several laboratories have identified the endocannabinoid signaling pathway, comprised of the endocannabinoid agonist anandamide and its pharmacological targets, CB₁ and CB₂ cannabinoid receptors and associated anandamide receptors, as a physiological system with capacity to mitigate cardiovascular and cerebrovascular disorders through neuronal and endothelial actions. Variability in experimental stroke models and modes of outcome evaluation, however, have provoked controversy regarding the precise roles of endocannabinoid signals in mediating neural and/or vascular protection versus neurovascular damage. Clinical trials of the CB₁ antagonist rimonabant demonstrate that modulation of endocannabinoid signaling during metabolic regulation of vascular disorders can significantly impact clinical outcomes, thus providing strong argument for therapeutic utility of endocannabinoids and/or cannabinoid receptors as targets for therapeutic intervention in cases of stroke and associated vascular disorders. The purpose of this review is to provide updated information from basic science and clinical perspectives on endocannabinoid ligands and their effects in the pathophysiologic genesis of stroke. Particular emphasis will be placed on the endocannabinoids anandamide and 2-arachidonylglycerol and CB₁ receptor-mediated mechanisms in the neurovascular unit during stroke pathogenesis. Deficiencies in our knowledge of endocannabinoids in the etiology and pathogenesis of stroke, caveats and limitations of existing studies, and future directions for investigation will be addressed.

Keywords

2-arachidonylglycerol; anandamide; CB₁ receptor; endocannabinoid; ischemia; matrix metalloproteinase; stroke

I. Introduction & Background on Stroke

The term *stroke* encompasses a broad and diverse family of cerebrovascular disorders that eventuate in rupture and/or occlusion of blood vessels supplying the brain thus disrupting blood flow and jeopardizing oxygen and essential nutrient supply to vital cerebral tissues. These events terminate in various degrees of focal and penumbral tissue infarction with acute and/or long-lasting and often grave consequences. Two primary forms of stroke exist:

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ischemic and hemorrhagic. Ischemic stroke results from cerebral thrombosis, a blood clot that develops at the focal site of occlusion within the blood vessel, and/or cerebral embolism, a clot forming at a distant site in the circulation and traveling to the cerebral vasculature where it lodges in a vessel of smaller caliber. A common etiology for cerebral embolism is atrial fibrillation, a cardiac malfunction whereby clots form in the left ventricle, dislodge, and travel via carotid vasculature to the brain. These blood clots (or other entities including fatty deposits, cholesterol, phospholipids, macrophages and/or other inflammatory mediators) lodge within the vessel lumen and disrupt or prevent blood flow to downstream tissues, resulting in focal hypoxia and anoxia and ultimately tissue necrosis and infarct. Following the immediate localized necrotic response, the penumbral region surrounding the focal infarct undergoes delayed cell death. Another form of cerebral ischemia follows cardiac arrest which results in global reduction of blood supply to systemic vasculature including, critically, the brain. Ischemic brain tissue may undergo a complex multi-factorial process whereby it converts to a hemorrhagic lesion with vascular leakage, fluid extravasation, and expanded tissue injury [2, 3]. It is estimated that 30 to 40% of all ischemic strokes undergo spontaneous hemorrhagic transformation and these estimates significantly increase in patients on thrombolytic therapy [4, 5].

Hemorrhagic stroke is the result of intracerebral and/or subarachnoid bleeding into surrounding cerebral tissue thereby increasing intracranial pressure on specific portions of the brain and altering normal functions of these tissues. The degree and amount of bleeding in hemorrhagic stroke determines the severity of the symptoms and prognoses for the individual. Two types of weakened blood vessels normally contribute to the pathogenesis of hemorrhagic stroke: aneurysmal (a ballooning and thinning of a weakened region of the vessel wall) and arteriovenous malformation (a cluster of abnormally formed vessels). Between 80 and 85% of all strokes are ischemic in nature while 15–20% of strokes are hemorrhagic, yet hemorrhagic strokes are responsible for greater than 30% of all stroke deaths [1]. Each year, approximately 750,000 Americans suffer a new or recurrent stroke, and about 160,000 of these individuals will die as a result. Primary and secondary stroke and associated complications are the third leading cause of death in Americans after heart disease and cancer, and stroke-related medical and disability costs are expected to reach \$58 billion for 2006 [1]. Moreover, demographic data reveal higher stroke incidence rates and stroke-related mortality among African American compared to Caucasian populations [6, 7]. Indeed, the broad health and economic consequences of stroke mandate identification of therapies for prevention and management of these serious neurovascular disorders.

II. Background on Endocannabinoids

A. Structure

The first endogenous cannabinoid, anandamide (AEA), was identified in 1992 [8]. A second endocannabinoid, 2-arachidonoylglycerol (2-AG), was subsequently discovered in 1995 [9, 10]. The chemical structures of AEA and 2-AG are shown in Fig. (1). Both of these compounds are arachidonic acid derivatives that bind to CB₁ and CB₂ cannabinoid receptors with different affinities thereby evoking differential activation. During the last five years, several other bioactive lipid mediators have been described in the scientific literature that appear to act, at least in part, through CB₁ and/or CB₂ receptors and that confer specific pharmacological effects *in vivo* [11]. These compounds are *O*-arachidonylethanolamine (virodhamine) [12, 13], *N*-arachidonoyldopamine [14, 15], 2-arachidonoyl-glyceryl ether (noladin ether) [16, 17], and oleamide [13, 18, 19]. However, physiological functions for these latter compounds have not yet been studied in detail and warrant further investigation. In this section, discussion on the synthesis, release, uptake, and degradation mechanisms will be brief and limited to AEA and 2-AG. Detailed and comprehensive description of the biochemical mechanisms for AEA and 2-AG is beyond the scope of this current article and

the reader is referred to several excellent and exhaustive reviews on these topics [20, 21, 22].

B. Synthesis, Release, Uptake, and Degradation of AEA and 2-AG

Endocannabinoid AEA and 2-AG are lipophilic in nature and consequently cannot be stored in cytosolic vesicles like classical neurotransmitters. In general, the regulation of endocannabinoid signaling is controlled by steady-state turnover through complementary synthesis, release, uptake, and degradation processes [23, 24, 25]. Several different stimuli such as membrane depolarization and increased intracellular Ca^{2+} and/or receptor stimulation can activate complex enzymatic machineries leading to cleavage of membrane phospholipids, activation of arachidonic acid biochemistry, and endocannabinoid biosynthesis. Importantly, different enzymes are involved in the synthesis of distinct endocannabinoids, indicating independent involvement of endocannabinoids under varied physiological and/or pathophysiological conditions [20, 21, 22].

Following syntheses, AEA and 2-AG (as well as other endocannabinoids) can bind to cannabinoid receptors on the external face of the plasma membrane and instigate a signaling cascade or move directly from the extracellular space through the cell membrane into the cytosol via non-receptor-mediated mechanisms. Endocannabinoids are catabolized by efficient degradation processes involving facilitated uptake from the extracellular milieu into the cell and enzymatic degradation mediated by specific intracellular enzymes. The exact nature of the carrier proteins involved in endocannabinoid uptake and their associated mechanism(s) are not clearly understood; however, enzymes responsible for the degradation of endocannabinoids are well characterized and are known as fatty acid amide hydrolase (FAAH) for anandamide and related compounds [25, 26] and monoglycerol lipase for 2-AG [27, 28]. An important feature of endocannabinoid bioactivity is the rapid induction of their synthesis, receptor activation, and degradation in coordinated and highly complementary fashion. This suggests that the endocannabinoid system acts on demand with tightly regulated spatial and temporal selectivity.

C. Endocannabinoid- and CB_1 Receptor-mediated Signaling

Several mechanisms underlying endocannabinoid-mediated signaling have been reported in the scientific literature and will be summarized in this section. In the central nervous system (CNS), endocannabinoids can act as neurotransmitters that transfer information from one neuron to the next. In this physiological microenvironment, postsynaptically-released endocannabinoids travel to the presynaptic site where they activate neuronal cannabinoid receptors (CB_1) and thus mediate signals in retrograde fashion [29, 30]. The overall effect of such a mechanism is a reduction in the release of neurotransmitters such as glutamate and gamma-aminobutyric acid (GABA). This phenomenon is present in synaptic connections of many brain regions and represents an important modulatory mechanism of neuronal transmission [30, 31, 32, 33]. A second mechanism of endocannabinoid-mediated signaling has been observed in GABAergic neurons in the cerebral cortex whereby endocannabinoids mediate autocrine signaling that induces self-inhibitory effects on neuronal activity [34, 35, 36]. Thirdly, endocannabinoids may act in paracrine or autocrine manner irrespective of synaptic transmission. This presumably occurs in glial cells and in non-neuronal cells such as adipocytes and hepatocytes [30]. Lastly, because endocannabinoids and CB_1 receptors are present in the intracellular environment, it cannot be excluded that endocannabinoids act as intracellular signaling molecules. Importantly, AEA and 2-AG do not appear as interchangeable mediators and indeed affect distinct regions within the brain. For example, electrophysiological and biochemical evidence shows that 2-AG is mostly involved in retrograde control of synaptic activity in the hippocampus or the VTA [37], whereas AEA appears to play an important roles in other cerebral regions such as the basal ganglia [30, 38]

and the amygdala [30, 39]. Thus, endocannabinoids and particularly AEA and 2-AG appear to be extremely versatile signaling mediators that are involved in a broad spectrum of physiological and pathophysiological regulatory processes.

Delta-9-tetrahydrocannabinol (Δ^9 -THC) and other cannabinoid agonists (e.g., HU210, WIN55,212-2, CP55940) are known to exert neurobehavioral and immune effects through cannabinoid receptors. To date, two cannabinoid receptor subtypes have been identified. The first cannabinoid receptor, CB₁, was originally identified in rat brain [40] and cloned [41, 42] and subsequently identified in peripheral tissues as well [43]. The second cannabinoid receptor subtype, CB₂, was identified and cloned from HL60 pro-myelocytes [44] and has been identified in brain [45] and immune cells including microglia [30, 46]. Both CB₁ and CB₂ receptors are coupled to G_{i/o} proteins and inhibit adenylyl cyclase, immediate early gene induction, and activation of mitogen activated protein kinases (MAPK) [47, 48]. Both CB₁ and CB₂ receptors exert constitutive basal activities that can be inhibited by inverse agonists such as rimonabant (SR141716) and SR144528, respectively.

The endocannabinoid system provides a novel mechanism through which to develop improved therapies for cardiovascular and neurological disorders. Clinical trials with the CB₁ antagonist rimonabant, namely RIO-Lipid and RIO-Europe, demonstrate that intervention in the endocannabinoid system can significantly improve clinical outcomes under conditions of cardiovascular adversity [49, 50]. In these clinical studies, 20 mg/day rimonabant produced significant decreases in body weight and waist circumference in obese individuals and improved their lipid profile and ability to regulate glucose. Furthermore, the frequency of metabolic syndromes was reduced with rimonabant treatment. Preclinical data have also demonstrated a long duration of action for rimonabant, and adverse drug-drug or drug-food interactions have yet to be reported. Since CB₁ has been implicated in the etiology and progression of diverse complications, these reported beneficial effects of rimonabant raise its candidacy for use as a future therapeutic aid.

III. Matrix Metalloprotease Regulation in Stroke

Various cell types are involved in the pathogenesis of cerebral infarction and include brain cells and cerebral nerves, inflammatory cells, vascular smooth muscle cells (VSMCs), and vascular endothelial cells (VECs). In cerebral nerves and surrounding brain vasculature, diverse cellular and molecular events contribute to stroke pathogenesis including important alterations in matrix-degrading matrix metalloproteinases (MMPs) [51, 52]. The MMPs constitute a family of over 25 zinc-dependent proteolytic enzymes that act upon a spectrum of intracellular, extracellular, and membrane-associated substrates and exert numerous and varied biological processes. In cerebral and systemic blood vessels MMPs are involved in the degradation of basal lamina and extracellular matrix (ECM) components and are essential for cell proliferation and cell migration [53, 54, 55], critical events that underlie normal growth as well as the pathophysiologic responses to an array of vascular dysfunctions including stroke. MMPs are also intimately involved in the regulation of atherosclerotic plaque and in part regulate plaque evolution, stability, integrity and complication [56, 57, 58]. In this context, alterations in matrix balance via complementary actions of MMPs and their extracellular tissue inhibitor of MMPs (TIMPs) directly control fibrous cap integrity and dictate risk from plaque progression, rupture, and/or erosion. Similarly, degradation of the ECM of the blood-brain barrier (BBB) contributes to increased permeability and cerebral hemorrhage. Understandably, regulation of matrix balance with particular emphasis on MMP chemistry and biology is suggested as a promising therapeutic approach for the treatment of a wide variety of cardiac, vascular, and neural complications including atherosclerosis, neoplastic disorders, and stroke [59, 60].

Complex and integrated mechanisms control MMP activity, primarily at the transcriptional and post-translational levels, and include proteolytic activation of inactive MMP zymogens, protease-, membrane-associated, and/or extracellular MMP-inducer protein (EMMPRIN)-mediated stimulation [61], direct inhibition by extracellular TIMPs, and influence by numerous growth factors, cytokines, and chemokines. This complexity allows for selective control of specific MMP-dependent parameters that serve to regulate matrix balance and biology and directly impact vascular physiology and disease pathogenesis. Most MMPs are produced intracellularly as immature (incompletely active) zymogens and are secreted as latent pro-enzymes that require extracellular cleavage to attain full biological activity [62, 63]. Step-wise activation of MMPs proceeds through disruption of the cysteine-zinc contact (ie., cysteine switch) that lies adjacent to the catalytic site by means of proteinase action (or by chemical agents, acidosis, and/or elevated heat under in vitro conditions), thereby allowing proteolytic removal of the pro-peptide region and intermolecular processing by partially-activated MMP intermediates or fully active MMPs [62]. Many MMPs are activated by serine proteases such as plasmin, although MMP-2 (72 kD gelatinase/gelatinase A) is uniquely activated at the cell membrane by a complex interaction between membrane-type MMPs (MT-MMPs) and TIMP-2 [62, 63]. Once activated in the extracellular milieu, MMPs can act upon a wide variety of substrates including principally components of the ECM (collagens, proteoglycans, fibronectin) and ECM-residing growth factors, inflammatory mediators, and factors in the apoptotic cascade. Moreover, non-ECM-associated proteins can serve as substrates for numerous MMP actions [64], thus markedly expanding the biological significance of this important family under homeostatic as well as pathologic conditions.

In neurons of the adult brain basal MMP expression is normally low but has been identified for MMP-2, -3, -7, -8, -9, -10, -11, -12, -13, -14, -15, and -16 [65]. Of these, the gelatinolytic enzymes MMP-2 and MMP-9 (92 kD gelatinase/gelatinase B) have been the most widely studied and characterized. Moreover, these gelatinases are subject to significant up- or down-regulation following induction, are firmly implicated in the multi-factorial pathogenesis of stroke, and often serve as basis for comparison to other MMPs. In general, following cerebral ischemic or hemorrhagic insult focal MMP systems are modulated in context-specific manner in an array of cell types including VECs, microglia, oligodendrocytes, neutrophils, neurons, and astrocytes. Specific gelatinase activities were identified in a seminal study by Rosenberg and colleagues [66] following experimentally-induced focal ischemia by middle cerebral artery occlusion (MCAO) in rats. Temporal gelatinase activities were observed in this study as the induction of MMP-9 preceded that of MMP-2 in the infarcted region. The authors concluded that MMP-9 must be involved in the acute responses to tissue damage while MMP-2 is significant in subsequent tissue repair mechanisms. Using the same experimental model, investigators found that both MMP-2 and MMP-9 are induced early with sustained expression that persists for several days [67]. Acutely, MMP-9 was localized to VECs and infiltrating neutrophils resident within the infarcted area as well to penumbral regions surrounding the infarcted area. In distinct fashion compared to MMP-9, MMP-2 activity was significantly increased in infiltrated macrophages only after 24 h and was maximized 5 days following MCAO [67]. The decrease in infarct size or reduction in vasogenic edema with systemic administration of a MMP-9 neutralizing monoclonal antibody [67] confirmed a role for MMP-9 in the deleterious effects of ischemic stroke. Additional studies verified activities of both MMP-2 and MMP-9 following experimentally-induced cerebral ischemia in rats [68, 69, 70] and identified involvement of the extracellular MMP-inducer protein EMMPRIN [68] and tissue plasminogen activator (tPA) [71] as associated mechanisms in mediating stroke-induced responses. Several other notable studies have been published recently that focus on involvement of MMP-9 during stroke pathogenesis. Extracellular proteolysis via MMPs and particularly MMP-9 was shown to be critical for disruption of an intact BBB and increasing

vascular permeability and cerebral edema, pathological steps that often ensue in fulminant neuroinflammatory responses [61, 72]. Cellular and biochemical studies show that increased expression and activation of MMPs contributes to BBB rupture and succeeding hemorrhagic cascades [73, 74, 75]. The degradation of proteins of the ECM within the neurovascular unit by MMPs has been suggested to be critical in the initiation and progression of neuronal death following stroke [61, 76, 77]. The findings that intracerebral injection of MMP-2 resulted in the opening of the BBB and formation of hemorrhage around the blood vessels, and co-administration of TIMP-2 inhibited this response [75], confirmed a role for MMP-2 in BBB disruption during stroke. The blockade of the opening of the BBB by systemic administration of a MMP-9 inhibitor [61, 78, 79, 80, 81] or reduction in BBB damage in MMP-9 knock-out mice after focal ischemia [78], and reported findings that MMP expression increases progressively over time following cerebral ischemia compared to non-ischemic controls [67, 75, 80, 82], strongly argue for deleterious involvement of gelatinolytic MMPs in stroke-related damage.

Apoptotic actions of MMP-2 and MMP-9, via induction of caspase-mediated cell death [70] or through involvement of the cysteine proteases calpain and cathepsin B [83], have also been implicated in the process of extracellular proteolysis underlying BBB disruption and cerebral hemorrhage. Complementary studies substantiate regulatory roles for MMP-9 in the pathogenesis of stroke and speculate that this target could serve as a viable therapeutic endpoint [51, 61, 84, 85, 86]. Regarding potential therapeutic intervention, it is essential to note that temporal regulation of matrix balance by MMPs is an important consideration, as protective effects of acute MMP inhibition do not necessarily correlate with delayed involvement of MMPs that are suggested to serve beneficial effects by promoting stroke recovery [84].

In addition to the gelatinases MMP-2 and MMP-9, MMP-13 (also known as collagenase 3) is intimately involved in degradation of collagen type II, gelatin, aggrecan, and fibronectin in a variety of tissues including cerebral neurons and is suggested to play a role in the pathogenesis of stroke. Of clinical importance, MMP-13 has been implicated in the etiology of vascular aneurysms, suggesting a role for this MMP in destructive remodeling of vascular ECM [87] and BBB dysfunction. Additionally, a polymorphism in the upstream MMP-13 promoter is theorized to be a genetic risk factor for enhanced fibrous plaque formation in African American males [88]. After experimental MCAO followed by blood reperfusion (MCAO/R), MMP-13 is highly induced in cerebral ischemic neurons and is suggested to play a pivotal role in ischemia-induced peri-neuronal matrix remodeling and reorganization [89]. Active involvement for the TIMP family of MMP inhibitors in the pathology of cerebral ischemia is also been suggested [61, 62, 63]. Four TIMPs have been identified in the adult brain and are specific endogenous inhibitors that bind particular MMPs in a 1:1 stoichiometry, thereby directly affecting MMP activities. A comprehensive knowledge of TIMPs in stroke pathogenesis is important for complete understanding of matrix regulation in the etiology of stroke yet is outside the scope of the current review.

IV. Currently Proposed Mechanism(s) for Endocannabinoids in Stroke

Evidence for the involvement of the endocannabinoid system in the pathophysiology of ischemic stroke is just emerging. Tissue concentrations of endogenous cannabinoids AEA and 2-AG were found to be increased in the lesioned brain areas in rat models of cerebral ischemia [74]. In this study, MCAO-induced focal cerebral ischemia resulted in significant increases in AEA and other *N*-acylethanolamines in the infarcted hemisphere compared to non-infarcted regions of the brain. In another study an increase in CB₁ receptor density was observed following focal ischemia but no increases in endocannabinoid levels were detected [90]. It is noteworthy that there are at least two reports of increases in AEA levels with no

detectable changes in 2-AG levels following ischemia [91, 92]. This finding is in sharp contrast to results using a hepatic ischemia-reperfusion model [93] or an experimental acute myocardial infarction model [94], in which AEA and 2-AG levels increased simultaneously in coordinated fashion. The ischemic injury-induced elevations in endocannabinoid content, together with evidence that activation of the CB₁ receptor reduces CNS excitability, implies that the endocannabinoid system comprises an endogenous protective mechanism during CNS injury [95, 96].

In an in vivo model of global ischemia, pre-treatment with the CB₁ agonist WIN55,212-2 protected hippocampal neurons from death after 3 days of ischemia [97]. CB₁ receptor knock-out mice were reported to develop severe neurological deficits following transient focal cerebral ischemia along with decreased cerebral blood flow in the ischemic penumbra compared to wild type littermates [98]. Results from these studies indicate that the CB₁ receptors provide endogenous neuroprotection following neuronal insult. Conversely, results from Berger and colleagues [91] using a permanent MCAO model of ischemic stroke showed that pre-treatment with the CB₁ antagonist rimonabant significantly reduced (40%) infarct volume at 5 h after injury. Analogous results were reported from Hillard's laboratory [74] in a rat transient focal cerebral ischemia model where pre-treatment with rimonabant significantly reduced (50%) ischemia-induced infarct size 24 h after transient (2 h) occlusion. Results from these studies imply that endogenous activation of CB₁ signaling can be deleterious to the outcome of CNS injury. Indeed, the abundance of discordant results describing protective versus deleterious effects of the endocannabinoid/CB receptor system demands further study and characterization.

V. Plausible New Mechanisms for Endocannabinoids in Stroke

The endocannabinoid AEA has been shown to stimulate endothelial nitric oxide synthase (eNOS) and produce nitric oxide (NO) in VECs [99, 100], in VSMCs [101, 102, 103], and in invertebrate neural ganglia and astrocytes [103, 104, 105]. Endothelial NOS- and neuronal NOS (nNOS)-derived NO has been firmly implicated in the activation of MMPs in endothelial, neuronal and microglial cells that constitute the neurovascular unit [102, 106, 107, 108, 109, 110, 111, 112]. Consequently, it seems highly plausible that endocannabinoid-mediated production of NO is capable of regulating MMP biology in the endothelial, neuronal and/or glial cells within the neurovascular unit. A recent report shows that the endocannabinoid AEA analog methanandamide increases mRNA expression and activity of MMP-1, MMP-3 and MMP-9 in human non-pigmented ciliary epithelial cells [113]. Corresponding new findings from our laboratories show that in a cultured bovine brain microvascular endothelial cell (BMEC) model, CoCl₂-induced hypoxia produced significant increases in the activities of MMP-9 and MMP-2 in time-dependent fashion as observed in the gel zymogram shown in Fig. (2). Interestingly, co-treatment with methanandamide (1 μ M) significantly attenuated the CoCl₂-mediated increase in MMP-9 activity and marginally reduced MMP-2 activity as well. These important findings suggest that endocannabinoid signaling via methanandamide inhibits hypoxia-induced MMP-9 and MMP-2 activities. These novel discoveries, along with evidence of immediate increases in endocannabinoid concentrations and CB₁ receptor expression following experimentally-induced ischemic stroke, strongly suggest that endocannabinoids acting on CB₁ cannabinoid receptors in the neuronal, endothelial, and glial cells and platelets of the neurovascular unit have capacity to regulate MMP biology and to significantly influence ensuing pathophysiologic responses.

The signaling mechanism of CB₁-mediated MMP regulation is poorly understood. Research in this particular area is still in its early phases and results are just emerging. Although exact mechanisms are not clear, the following discussion describes plausible signal transduction

pathways for CB₁ cannabinoid receptor-mediated MMP regulation. In rat B103 neuroblastoma cells, anandamide-mediated activation of CB₁ receptors resulted in cell rounding via a pertussis toxin (PTX)-insensitive and Rho kinase inhibitor-sensitive process [114]. These results suggest that besides Gi/o proteins, the CB₁ receptor is also coupled with G proteins (possibly G_{12/13}) that activate small G-proteins such as Rho which has been implicated in the regulation of MMP activity. In another study, CB₁ activation by the synthetic agonist WIN55,212-2 significantly decreased neo-angiogenesis in granuloma tissue and inhibited nuclear factor-kappa B (NF- κ B)/DNA binding activity [115]. The decrease in NF- κ B activity was associated with a decrease in inducible nitric oxide synthase (iNOS)-mediated NO production. Nitric oxide has been implicated in the production and activation of MMP in many different cell types including endothelial, neuronal and glial cells [102, 106, 107, 108, 109, 110, 111, 112]. Activation of the CB₁ receptor has also been shown to inhibit the VEGF pathway [116, 117, 118], while activation of the VEGF receptor stimulates MMP activity in many cell types and thus, trans-regulation of the VEGF pathway could act as a means for CB₁ receptor-mediated MMP regulation. In summary, the CB₁ receptor is emerging as an important therapeutic target in the genesis and pathophysiology of ischemic stroke and associated neural and/or vascular complications.

VI. Future Directions for Investigation

Emerging experimental evidence strongly suggests that endocannabinoids and in particular AEA and 2-AG as well as the endocannabinoid receptor CB₁ can serve as pivotal signaling mediators of normal physiological functions as well as bioactive factors during the pathogenesis of cerebral stroke. Discordant data, however, regarding endocannabinoid-mediated neuroprotection following cerebral ischemia exist and exact molecular and cellular mechanisms are still not clearly defined. Reported effects of pharmacological blockade of CB₁ receptors versus genetic ablation of CB₁ receptors are conflicting; moreover, it is plausible to speculate that effects of endocannabinoids and CB₁ receptor antagonists in the regulation of neuroprotection following ischemic stroke occur via CB₁ receptor-independent avenues [119, 120]. Alternatively, endocannabinoid-induced NO might play significant roles in the regulation of acute MMP activity and subsequent neuronal infarction (immediately after stroke as well as delayed MMP activity) and serve salutary effects toward constructive plasticity and remodeling in stroke recovery [84]. The influence of NO-derived reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) and their association with endocannabinoids in the etiologies of stroke and associated vascular and neural pathologies is of critical importance and the reader is referred to an excellent updated review on this topic [121]. Clearly, further research in these areas is highly warranted in order to fully evaluate the potential usefulness of the endocannabinoid system in stroke pathogenesis and therapy.

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List of Abbreviations

2-AG	2-arachidonoylglycerol
AEA	anandamide

BBB	blood-brain barrier
BMEC	bovine brain microvascular endothelial cell
Ca²⁺	calcium
CNS	central nervous system
CP55940	5-(1,1-Dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)-cyclohexyl]phenol
ECM	extracellular matrix
EMMPRIN	extracellular matrix metalloproteinase-inducer protein
eNOS	endothelial nitric oxide synthase
FAAH	fatty acid amide hydrolase
GABA	gamma-aminobutyric acid
h	hour
HU-210	1,1-Dimethylheptyl-11-hydroxytetrahydrocannabinol
iNOS	inducible nitric oxide synthase
MAPK	mitogen activated protein kinases
MCAO	middle cerebral artery occlusion
MCAO/R	middle cerebral artery occlusion/reperfusion
MMP	matrix metalloproteinase
MT-MMP	membrane-type matrix metalloproteinase
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NF-κB	nuclear factor-kappa B
PTX	pertussis toxin
ROS	reactive oxygen species
RNS	reactive nitrogen species
SR141716	rimonabant
Δ^9-THC	delta-9-tetrahydrocannabinol
TIMP	tissue inhibitor of matrix metalloproteinase
tPA	tissue plasminogen activator
VEC	vascular endothelial cell
VSMC	vascular smooth muscle cell
VTa	ventral tegmental area
WIN55,212-2	(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone

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Fig. (1).
Chemical structures of anandamide and 2-arachidonoylglycerol.

Fig. (2).

Bovine brain microvascular endothelial cells were grown in EBM media (Clonetics) with recommended growth factors to 90% confluence and then subjected to CoCl_2 -induced hypoxia for 0, 4 or 8 h in the presence of vehicle or the endocannabinoid analogue methanandamide and 0.1% fetal bovine serum. Secreted MMP activities were assessed using 10% SDS-PAGE gelatin (0.1%) zymography. Results clearly demonstrate that methanandamide reduces CoCl_2 -mediated elevations in the extracellular activities of MMP-2 and MMP-9.