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Using the endocannabinoid system as a neuroprotective strategy in perinatal hypoxic-ischemic brain injury

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Abstract

One of the most important causes of brain injury in the neonatal period is a perinatal hypoxic-ischemic event. This devastating condition can lead to long-term neurological deficits or even death. After hypoxic-ischemic brain injury, a variety of specific cellular mechanisms are set in motion, triggering cell damage and finally producing cell death. Effective therapeutic treatments against this phenomenon are still unavailable because of complex molecular mechanisms underlying hypoxic-ischemic brain injury. After a thorough understanding of the mechanism underlying neural plasticity following hypoxic-ischemic brain injury, various neuroprotective therapies have been developed for alleviating brain injury and improving long-term outcomes. Among them, the endocannabinoid system emerges as a natural system of neuroprotection. The endocannabinoid system modulates a wide range of physiological processes in mammals and has demonstrated neuroprotective effects in different paradigms of acute brain injury, acting as a natural neuroprotectant. The aim of this review is to study the use of different therapies to induce long-term therapeutic effects after hypoxic-ischemic brain injury, and analyze the important role of the endocannabinoid system as a new neuroprotective strategy against perinatal hypoxic-ischemic brain injury.

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Research Highlights

(1) A perinatal hypoxic-ischemic event is one of the most important causes of brain injury in the neonatal period.
(2) Hypoxia/ischemia leads to brain cell damage, finally resulting in brain cell death.
(3) The complex molecular mechanisms underlying hypoxic-ischemic brain injury cause unsatisfactory efficacy of treatments for this condition.
(4) A thorough understanding of the mechanisms underlying hypoxic-ischemic brain injury will provide new insights for the development of novel neuroprotective agents for this condition.
(5) The endocannabinoid system, which is naturally neuroprotective, is likely to play an important role in the prevention and treatment of hypoxic-ischemic brain injury.

INTRODUCTION

Despite important advances in obstetric and neonatal care over the last 10 years, perinatal hypoxic-ischemic events still lead to significant mortality and morbidity in neonates. These events are one of the most important causes of neonatal brain injury and also result in adverse developmental outcomes[1-2]. The severity of the event, which often results in a dyskinetic cerebral palsy, is associated with multiple handicaps and hence a need for aid from society. Since hypoxic-ischemic brain injury is often unpredictable, the primary approach is to develop post-insult therapies to ameliorate ongoing or secondary injury[3]. In this regard, recent studies have focused on current knowledge surrounding neuroprotective therapies targeted towards the complexities of perinatal hypoxic-ischemic brain injury.

This article provides a comprehensive summary of the biochemical mechanisms underlying a perinatal hypoxic-ischemic event and a description of the morphological and molecular aspects of hypoxic-ischemic brain injury in neonates during intrauterine asphyxia.

Neuroprotective research has focused on pre-clinical studies of therapies that might reduce hypoxic-ischemic lesions to increase the opportunities of neonatal survival. Among them, cannabinoid compounds appear as a new therapeutic strategy with pharmacological properties for treatment of hypoxic-ischemic brain injury.

HYPOXIC-ISCHEMIC BRAIN INJURY

Hypoxic-ischemic encephalopathy, which is one of the most important causes of disabilities in term-born infants, leads to devastating long-term effects in the development of children[4]. The incidence of perinatal asphyxia ranges between 0.5–1% of all live births[5] and significant neurologic damage occurs in as many as 50–75% of these children[6]. Deficits include a variety of sensorimotor and cognitive impairments, depending on the extent, nature and location of the injury, as well as gestational age. These problems are encountered throughout development with a tremendous impact on the child, family, and society[7-8]. Despite the improvements in perinatal care, developmental neurological disorders are still a noteworthy problem[9].

It is necessary to take into account that the preterm neonate brain is more susceptible to hypoxic-ischemic events than the adult brain. Its cerebral vasculature makes the preterm neonate particularly vulnerable to periventricular and intraventricular hemorrhage. The preterm brain has more blood vessels, higher water content, lower myelin, a poorly developed cortex and a prominent germinal matrix[8]. Moreover, because of the vulnerability of immature oligodendrocytes in the white matter and the disruption in the myelination of motor tracts, common disorders, such as spastic paresis of the lower extremities, dyskinetic cerebral palsy and visual impairments, develop in children suffering hypoxic-ischemic brain injury[10-16].

To improve care in perinatal asphyxia, it is necessary to focus on the period of time following the hypoxic-ischemic event where therapeutic strategies could be efficacious in reducing brain injury. This period is normally short and may vary from 2 to 6 hours. Therefore, rapid identification would facilitate the application of diverse rescue strategies. To reduce the neurological consequences derived from hypoxic-ischemic brain injury, some actions are required: (1) improved monitoring in the perinatal period; (2) rapid identification of affected neonates; (3) preconditioning therapy (a therapeutic method that reduces the brain vulnerability) before hypoxic-ischemic encephalopathy; and (4) prompt institution of post-insult therapies to ameliorate the evolving injury[17-18].

BIOCHEMICAL AND PHYSIOLOGICAL EVENTS FOLLOWING HYPOXIC-ISCHEMIC BRAIN INJURY

The principal pathogenetic mechanism underlying neurological damage resulting from hypoxia-ischemia comprises a biphasic pattern of damage. An initial phase of early energetic failure is then followed by late energetic failure and occurs during reperfusion and reoxygenation several hours after the initial insult, and can last for days. The main cause of hypoxic-ischemic brain injury is the deprivation of glucose and oxygen, which results in a primary energy failure (first phase) and initiates a cascade of biochemical events leading to cell dysfunction and ultimately cell death[19-22].

During early energetic failure, the decrease in oxidative energy metabolism generates significant impairment in the extracellular balance of glutamate. Glutamate accumulation at synapses activates N-methyl-D-aspartate receptors. The uncontrolled stimulation of

these receptors can lead to neuronal death through a process called excitotoxicity[21, 23]. The subsequent release of excitatory amino acid transmitters and the formation of toxic free-radicals trigger a metabolic cascade on a slower time scale, leading to an influx of Ca^{2+} into neurons and promoting necrotic cell death. In this sense, necrosis is generated by organelle and cell swelling (cerebral edema), followed by the rupture of the plasma membrane and finally the dissolution of cell membranes in a zone surrounding the irreversibly damaged infarct core[23-26].

The secondary energy failure (second phase) varies according to the nature of the insult. High-energy phosphate levels recover from baseline levels[8, 27-28] after reperfusion and a second decline in high-energy phosphate levels is pronounced in the next 48 hours[29-31]. This secondary phase is characterized by excessive entry of Ca^{2+} into cells, causing induction of free radicals, such as reactive oxygen species. Excitotoxic amino acids are also released and inflammatory reactions occur in the immediate zone surrounding the infarct *i.e*., the penumbra. These reactions promote cell death, mainly by apoptotic mechanisms including shrinkage[32-36], nuclear pyknosis (the nucleus loses density), chromatin condensation, and genomic fragmentation[37] (Figure 1).

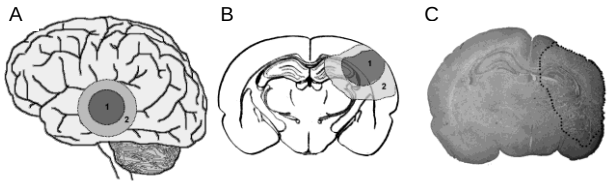

Figure 1 Schematic distribution of brain injured regions with different severity of hypoxia-ischemia (HI).

(A) Lateral and (B) coronal diagrams of the brain at interaural distance 5.40 mm and Bregma –3.60 mm. Area 1 represents the irreversibly damaged tissue (core of lesion). Area 2 comprises the penumbra. In the central core, blood flow deficits and/or hemorrhagic lesions are severe and brain cells die rapidly. In the penumbra, cell death, inflammation, and neurovascular perturbations proceed at a slower pace[177]. Hence, it may be theoretically possible to salvage cells in this region following treatment.

(C) Representative photograph of perinatal rat brain 7 days after HI brain injury. The dotted line indicates the main affected areas after HI brain injury, which compromises the hippocampus and the ipsilateral cortex. Loss of tissue in this region is apparent.

This penumbral zone is characterized by a longer tolerance to ischemic stress and a slower rate of disintegration, due to a moderate metabolic derangement and minor abnormalities in cerebral blood flow[24, 26]. This biphasic pattern produced during the hypoxic-ischemic event (Figure 2) creates a “therapeutic window”, a period in which the damaged but viable cells could be rescued by neuroprotective strategies. This window attracts much interest as a target for therapeutic strategies to reduce the damage derived from hypoxic-ischemic insults.

IMMATURE BRAIN PLASTICITY AFTER HYPOXIC-ISCHEMIC BRAIN INJURY

Loss of cerebral function after hypoxic-ischemic brain injury is not only due to neuronal death in the infarcted tissue but also cell dysfunction in the penumbra. Therefore, it is important to take into account these surrounding penumbral areas that could survive the insult, as well as the non-ischemic ipsilateral tissue and the contralateral brain areas, which are connected to the area of damage[38-40].

Following hypoxic-ischemic brain injury, the neonatal nervous system is capable of making compensatory reorganization. This recovery depends on the severity, intensity and timing of injury[21, 41-42]. This spontaneous reorganization occurs during a period proximate to injury and probably reflects the recovery of neurotransmission in tissue near to and distant from the injury location[43-45]. Therefore, affected neurons that are damaged by catabolic processes could be rapidly repaired by the neonatal nervous system[46]. However, these neurons might still exhibit aberrant neurotransmission, due to the presence of dysfunctional spines[47-48].

Immature brain has demonstrated a particularly strong capacity to recover from hypoxic-ischemic brain injury by producing neurogenesis in non-neurogenic vulnerable regions to ischemic injury, and in this way new neurons produced in the subventricular zone can migrate to injured areas in the neocortex[49-50]. This neocortical neuron migration, which has great importance for the remyelination of injured areas, also guarantees the survival of the new neurons[49], and coincides with the proliferation and migration of glial cells[51]. Furthermore, survival, repair and plasticity genes are rapidly reactivated after ischemia in response to damage, and growth-promoting factors are released to stimulate anabolic processes[52-53]. These remarkable observations suggest the brain has the potential to repair itself, which is relevant to therapies for various pathological conditions, such as ischemic brain injury.

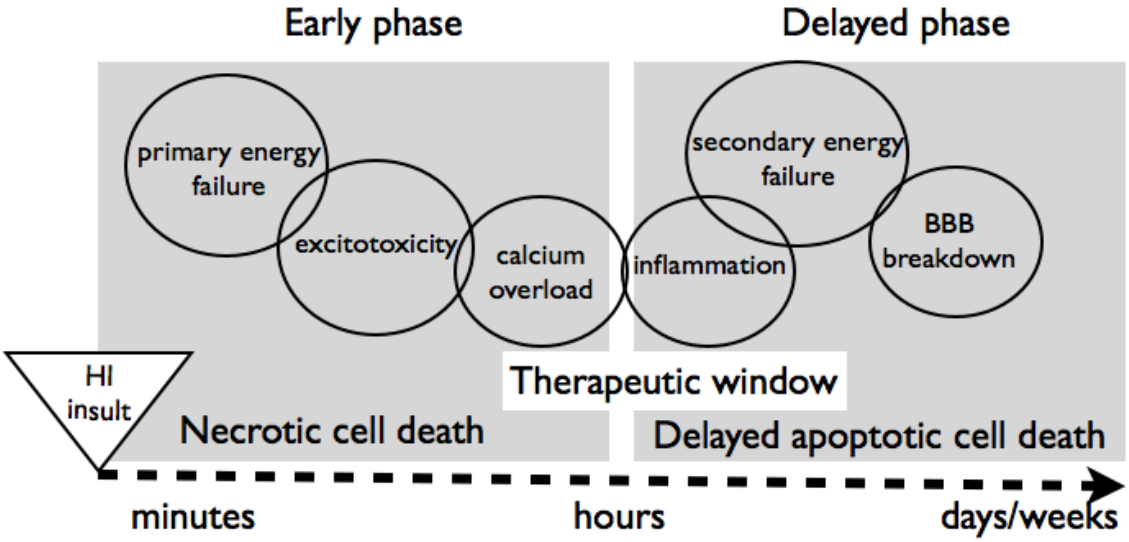

Figure 2 Cascade of biochemical reactions following hypoxic-ischemic (HI) brain injury[178].

Over a period of time, an HI event displays a biphasic pattern of cell death. A first phase of early energetic failure decreases the oxidative energy metabolism of cells and promotes necrotic cell death. A second phase of late energetic failure occurs during reperfusion and reoxygenation several hours after the initial insult and can last for days. The "therapeutic window", the period between these two phases, is a time when the damaged but live cells could be rescued by neuroprotective strategies. This window has been increasingly studied to allow for the development of therapeutic studies to reduce the damage derived from HI insult. BBB: Blood-brain barrier.

Furthermore, delayed regeneration is possible because neural stem cells can renew and differentiate themselves into cells of all glial and neuronal lineages and can populate developing or degenerating central nervous system regions. Recent evidence suggests that hypoxic-ischemic brain injury can also be treated with mesenchymal stem cells[54], which are easily recovered from bone marrow, placental tissue, umbilical cord stroma and cord blood without ethical issues. Mesenchymal stem cells may also secrete several trophic factors including stimulating factor-1, vascular endothelial growth factor, basic fibroblast growth factor, nerve growth factor and brain-derived neurotrophic factor[55]. Intracranial administration of mesenchymal stem cells for 3–10 days after hypoxic-ischemic insult has shown decreased histological damage and improved outcome in rat models of hypoxic-ischemic brain injury[56].

THERAPEUTIC STRATEGIES FOR HYPOXIC-ISCHEMIC DAMAGE

Reducing neuronal death after oxygen deprivation has provided promising results in experimental therapy, especially in the developing nervous system[57-58]. The main strategies related to amelioration of injury after hypoxia-ischemia are those applied after the insult or reperfusion[18, 59]. In this sense, non-pharmacological therapies, such as hypothermia, which consist of minimizing cerebral metabolism[60], have high relevance in clinical practice. However, many studies are now focusing on the use of pharmacological therapies to treat specific aspects of hypoxic-ischemic brain injury.

Non-pharmacological therapies

Hypercapnea and hypothermia stand out amongst the non-pharmacological therapies for the treatment of brain injury. In experimental assays in rats, hypercapnea has been reported to reduce lung injury, increase cerebral blood flow, and protect the immature brain from hypoxic-ischemic brain injury[61]. Currently, hypothermia appears to be the most reliable intervention available for reducing the risk of death or disability in infants with brain injury[62-63]. A moderate temperature reduction (32–34°C) has now become a standard of care for neonatal hypoxic-ischemic brain injury[64-65].

Results from MRI studies regarding hypothermia therapy suggest that head and total body cooling is associated with a decrease in the incidence of basal ganglia/thalamic brain lesions[16]. Mechanisms based on hypothermic neuroprotection are an increase in neuronal survival in the basal ganglia and suppression of

caspase-3 activation[66]. Hypothermia has also been shown to suppress microglial activation[67]. Furthermore, inflammation and expression levels of tumor necrosis factor-α, interleukin-1β and interleukin-18 are reduced[68] in this context, whereas an increase in the expression of the anti-inflammatory cytokine interleukin-10 has been observed[67, 69].

Pharmacological therapies

Recent studies have demonstrated that the administration of a variety of pharmacological agents after perinatal asphyxia is effective in alleviating injury. These specific drugs are used to reduce toxic free radicals, inhibit the excessive influx of calcium into neurons, and minimize cerebral edema caused by hypoxia-ischemia[7, 58, 70].

Regarding free radical formation after a hypoxic-ischemic event, allopurinol, a xanthine oxidase inhibitor, reduced the formation of free radicals that cause tissue damage and helped to maintain the blood-brain barrier. Its effectiveness has been noted in several animal studies[71-72]. Another alternative is the use of antioxidants such as erythropoietin, which has antiapoptotic and angiogenic properties[73] and provides neuroprotection and neurogenesis in neonatal rats[74-76]. By contrast, melatonin reduces brain damage and inhibits the development of long-term effects from ischemic injury[76], while vitamin E is thought to be an antioxidant and free radical scavenger, thereby reducing the risk and severity of hypoxic-ischemic brain injury[77].

Furthermore, administration of $MgSO_4$ has been suggested to act as a neuroprotective agent because magnesium ions block the N-methyl-D-aspartic acid receptors and can therefore act as potent antagonists of glutamate neurotoxicity[78]. Previous reports suggested that $MgSO_4$ administration prevented the effects of energy depletion after a hypoxic-ischemic event in newborn children[79], and altered important enzymes in erythrocyte membranes from asphyxiated newborns, reducing post-asphyxial damage[80]. Likewise, other authors explain how $MgSO_4$ has potential therapeutic benefits after the hypoxic-ischemic event, reducing the number of apoptotic cells[81].

Recently, several studies have indicated that cannabinoids have high potential as neuroprotective compounds, both in acute neurodegenerative diseases, such as hypoxic-ischemic or traumatic brain damage, and in chronic processes such as multiple sclerosis, Parkinson's disease and Alzheimer's disease[82-84]. These substances have emerged as neuroprotectants because they can modulate neuronal and glial responses. Additionally, cannabinoids have endothelial cell function, anti-excitotoxic[85-86], anti-inflammatory[86-88], and vasodilatory effects[89] and can also regulate calcium homeostasis[90-92]. New findings indicate that some anti-inflammatory treatments may actually improve recovery by promoting neurogenesis[92-93]. Cannabinoid receptor activation, therefore, is an important neuroprotective strategy for neonatal hypoxic-ischemic brain injury given its anti-inflammatory effect, with the synthetic cannabinoid WIN 55212 enhancing subventricular zone cell proliferation after neonatal hypoxic-ischemic brain injury[94].

NEUROPROTECTIVE EFFECTS OF THE CANNABINOID SYSTEM ON HYPOXIC-ISCHEMIC BRAIN INJURY

Up to now, different kinds of cannabinoid compounds have been described including phytocannabinoids, synthetic cannabinoids and those synthesized in the brain and certain peripheral tissues, named endogenous cannabinoids or endocannabinoids.

Phytocannabinoids

Phytocannabinoids are substances that normally have a carbocyclic structure of 21 carbons and are formed generally by three rings of cyclohexene, tetrahydropyran and benzene. These kinds of cannabinoids are produced by the cannabis plant, with the most representative molecules being tetrahydrocannabinol, cannabinol and cannabidiol[95].

Synthetic cannabinoids

The development of synthetic cannabinoids based on the chemical structure of phytocannabinoids has produced a large number of analogs. These compounds aimed to block the effects of the endogenous ligands by antagonizing cannabinoid receptors. For example, SR141716 or rimonabant[96] and AM251 are cannabinoid receptor type 1 receptor-selective antagonists, and SR144528[97] and AM630 are cannabinoid receptor type 2 receptor-selective antagonists. Alternatively, compounds can be designed to potentiate the effects associated with endogenous receptors through the use of specific agonists such as arachidonyl-2'-chloroethylamide (a cannabinoid receptor type 1 receptor-selective agonist), AM1241, JWH015 (a cannabinoid receptor type 2 receptor-selective agonists) or using non-selective agonists like CP55, 940[98], HU210

or WIN55, 212-2[99].

Endocannabinoids

Endocannabinoids are compounds produced within the body that have a lipid nature and are derived from polyunsaturated fatty acids with long chains. These compounds activate cannabinoid receptors and are synthesized in moments of intense activity of the brain[100]. The two most widely studied endocannabinoids are N-arachidonylethanolamide (anandamide)[101] and 2-arachidonoylglycerol[102-103]. Endocannabinoid names consist of the exogenous and endogenous ligands, the target receptors and the enzymes responsible for ligand biosynthesis, transport and degradation, such as N-acyltransferase, phospholipases, diacylglycerol lipase and the fatty acid amide hydrolase[104-106].

Endocannabinoid receptors

Endocannabinoids constitute a novel family of lipid ligands that act *via* specific G-protein-coupled receptors, cannabinoid receptor type 1 and cannabinoid receptor type 2[104]. Endocannabinoids can also interact with other receptors such as vanilloid receptors type 1[107], peroxisome proliferator-activated receptors and even the TWIK-related acid-sensitive potassium channel 1[108]. Moreover, some studies have also reported the interaction of anandamide with both muscarinic[109] and serotonergic receptors[110]. Cannabinoid receptor type 1 is widely expressed in neurons, with particularly high levels in the I and IV layers of the cerebral cortex, hippocampus, basal ganglia, cerebellum, and brainstem[104]. It can also be found in glial cells[95, 108, 111]. Such a distribution of cannabinoid receptor type 1 receptors suggests that cannabinoid agonists are involved in neuronal circuits related to coordination and modulation of movement, superior cognitive functions such as memory and reward mechanisms, response to stress and pain, regulation of sleep, body temperature, appetite, nausea and vomiting[107, 109-110]. The activation of presynaptic cannabinoid receptor type 1 has its main neuroprotective capacity in the inhibition of glutamatergic neurotransmission[111-114], and avoiding massive accumulation of intracellular calcium, nitrogen and reactive oxygen species, which would trigger cell death[115].

Cannabinoid receptor type 2 is expressed mainly in cells of lymphoid origin, such as B and T lymphocytes, natural killer cells, mastocytes, macrophages and monocytes. Therefore, they may be involved in the immunomodulatory effect of cannabinoids[95, 115-116]. Some reports have described the presence of cannabinoid receptor type 2 in brain cells, including neurons from the brain stem[105, 116-117]. Cannabinoid receptor type 2 mediates the cannabinoid anti-inflammatory and immunomodulatory effects[118-119], with a number of investigations showing that their activation has anti-inflammatory therapeutic potential in central nervous system diseases, such as multiple sclerosis, traumatic brain injury and Alzheimer's disease[120-121]. Recently, it has been shown that the cannabinoid receptor type 2 is also found in resident inflammatory cells within the brain, such as microglia[122-123], and that hypoxia-ischemia induces its expression in the brain[119]. Thus, the use of cannabinoid receptor type 2 agonists has proven to be beneficial in different paradigms of neonatal hypoxic-ischemic brain injury[124-126], by reducing cell death accompanied by modulation of glutamate release, and decreasing production of cytokines, cyclooxygenase-2 and inducible nitric oxide synthase expression. These observations support the hypothesis that the protective effect of cannabinoid receptor type 2 relies mostly upon its anti-inflammatory effects and opens a new possibility for its use as a neuroprotective target following perinatal asphyxia.

Endogenous ligands

As mentioned above, the best characterized endogenous ligands are N-arachidonoylethanolamide and 2-arachidonoylglycerol[101, 126-128]. N-arachidonoylethanolamide or anandamide has been found both in the brain and in the periphery[129]. Anandamide is an agonist for cannabinoid receptor type 1[130-131], cannabinoid receptor type 2[129, 131], and the vanilloid receptor type 1 receptor[132]. In the brain, anandamide levels are high in the hippocampus, thalamus, striatum and brainstem, and lower, but still detectable, in the cerebral cortex and cerebellum[129, 133]. Like anandamide, 2-arachidonoylglycerol is found both in the brain and in the periphery, although the concentrations found are approximately 150 times higher than those for anandamide[133]. 2-arachidonoylglycerol is found at high levels in the brainstem, hippocampus, striatum and medulla in rats, showing a correlation with anandamide but not cannabinoid receptor type 1 localization[133]. 2-arachidonoylglycerol is an agonist at cannabinoid receptor type 1 and also cannabinoid receptor type 2, having a greater potency than anandamide. This suggests that 2-arachidonoylglycerol may be the endogenous ligand for cannabinoid receptor type 2, and this finding could be due to the greater stability of 2-arachidonoylglycerol compared with anandamide[103, 134].

In addition, unknown or poorly investigated

endocannabinoids do exist. They are present in the mammalian nervous system but their exact physiological and pathological functional relevance is still obscure. *In vitro* assays demonstrated that some of these novel endocannabinoids act upon known cannabinoid receptors[135].

Endocannabinoid synthesis

The synthesis of endocannabinoids (Figure 3) results from an intense or prolonged release of excitatory neurotransmitters[136]. In response to postsynaptic depolarization, voltage-dependent calcium channels are opened, inducing an increase in intracellular calcium levels. This increase in intracellular Ca^{2+}stimulates the process of exocytosis. Neurotransmitter is released and then binds to the corresponding postsynaptic receptor, inducing the opening of Ca^{2+} channels; consequently, the postsynaptic neuron is depolarized. The increase of intracellular Ca^{2+} activates enzymes such as N-acyl-transferase, phospholipases A, C and D or diacylglycerol lipase. These enzymes synthesize endocannabinoids from membrane lipids including phosphatidylethanolamine or phosphatidylcholine. Once synthesized, the endocannabinoids leave the postsynaptic cells and activate cannabinoid receptor type 1 on presynaptic neurons. Through the activation of G proteins, Ca^{2+} presynaptic channels are inhibited, and neurotransmitter release is suppressed[137-138].

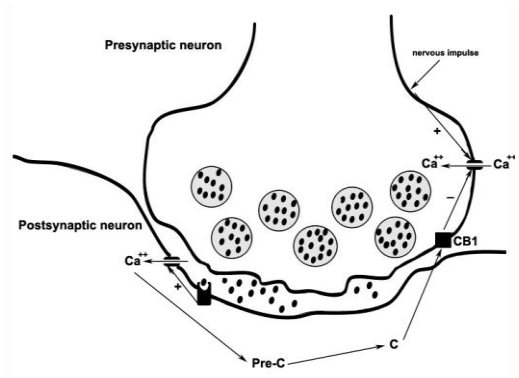

Figure 3 Role of cannabinoids in the modulation of synaptic transmission[169].

Presynaptic neuronal activation results in neurotransmitter release and induces an increase in intracellular calcium levels at the postsynaptic cell, thus activating anandamide and 2-arachidonoylglycerol production. Endocannabinoids act largely retrogradely and engage cannabinoid receptor type 1-mediated signaling pathways. CB1: Cannabinoid receptor type 1; C: cannabinoid; Pre-C: pre-cannabinoid.

Anandamide is produced on demand from the hydrolysis of a pre-formed membrane phospholipid precursor N-arachidonoyl phosphatidylethanolamine by the action of N-arachidonoyl phosphatidylethanolamine-phospholipase-D[139-140]. In most tissues, anandamide removal is catalyzed by fatty acid amide hydrolase[141], but can also act as a substrate for palmitoylethanolamide-preferring acid amidase[142-143], cyclooxygenase-2, lipoxygenases, and cytochrome P450 to produce biologically active products. 2-arachidonoylglycerol is also synthesized on demand through the conversion of 2-arachidonate-containing phosphoinositides to diacylglycerols, which are then converted to 2-arachidonoylglycerol by the action of diacylglycerol lipase. Monoacyl glycerol lipase is the enzyme mainly responsible for its metabolism *in vivo*, although it can be also be metabolized by fatty acid amide as well as by cyclooxygenase-2 and lipoxygenases[140-143].

Neuroprotective effects

The neuroprotective effect provided by cannabinoid receptor activation occurs because of the modulation of synaptic transmission[144-145], plasticity, calcium homeostasis[95, 103] and activation of cytoprotective signaling pathways[103].

It has been shown that endocannabinoids synthesized by depolarized postsynaptic dendrites, particularly 2-arachidonoylglycerol[146], can act as retrograde ligands at cannabinoid receptor type 1 located at presynaptic terminals to inhibit the release of excitatory or inhibitory neurotransmitters from the presynaptic neuron[147-148]. Moreover, endocannabinoids also play a key role in peripheral and brain immune function, including inhibiting the release of inflammatory mediators, such as nitric oxide, interleukin-2 and tumor necrosis factor-α. Endocannabinoids also inhibit the activation of cell-mediated immune processes, proliferation and chemotaxis[149-150].

Activation of cannabinoid receptors induces the closure of Ca^{2+} channels, thus inducing neuroprotection through the reduction of glutamate release[151-152]. Drugs reducing glutamate release are of particular value in neuroprotection in a neonatal hypoxic-ischemic event, as glutamate receptor blockers are neurotoxic in immature brains[4]. In addition, cannabinoids reduce direct N-methyl-D-aspartate toxicity by downstream inhibition of protein kinase A signaling and nitric oxide generation[153].

Several *in vitro* studies have reported neuroprotective effects of cannabinoids related to their antioxidant effect[154-155]. Also, *in vivo* models of neurodegenerative diseases have demonstrated antioxidant-related neuroprotective actions for cannabinoids[156]. It is known

that cannabinoids reduce body temperature[157]. Studies in adult rats using different cannabinoids have demonstrated that hypothermia is a substantial part of the neuroprotective effect of these compounds, as warming reduces or even abolishes the beneficial effect[158-159]. Furthermore, cannabinoids cause vasodilation in the brain[160-161], stabilize the blood-brain barrier and are involved in neuroproliferative processes[162-163]. Cannabinoids enhance energy metabolism of astrocytes[144] and protect these glial cells against cytotoxic and proapoptotic stimuli[164].

Different studies postulate that administration of synthetic cannabinoids can reduce damage after brain injury[165-170]. Specifically, administration of WIN55212 just after recovery from hypoxia-ischemia successfully reduces brain injury as observed in a histopathological study by Fernandez-Lopez *et al* [167]. Moreover, WIN55212 reduces apoptotic cell death in all regions studied through the maintenance of mitochondrial integrity and functionality[171] and promotes neurogenesis in the subventricular zone, oligodendrogenesis, white matter remyelination, and neuroblast generation after neonatal hypoxic-ischemic events[172]. Additionally, the cannabinoid receptor type 1 antagonist AM281 and the diacylglycerol-lipase inhibitor O-3640 have been shown to exacerbate the detrimental effects of oxygen-glucose deprivation in an *in vitro* model by causing an excess in glutamate release. The cannabinoid receptor type 2 agonist, O-1966, has been found to increase blood flow to the brain and thus attenuate neuroinflammation in an animal model of stroke[173].

Administration of endogenous cannabinoids emerges as a novel neuroprotective therapy because of the observation that these substances take part in the natural mechanism for controlling damage. According to their neuroprotective effects, experimental *in vitro* studies confirmed that the endocannabinoids anandamide and 2-arachidonoylglycerol may attenuate injury in cortical cells in an oxygen-glucose deprivation model[173]. In an *in vivo* model of induced excitotoxicity, anandamide protects against neuronal injury[174]. Moreover, in a mouse model of closed head injury, administration of 2-arachidonoylglycerol significantly reduced brain edema, infarct volume and hippocampal cell death, and promoted clinical recovery[175]. Finally, administration of these two endocannabinoids after perinatal hypoxic-ischemic brain injury in a rat model remarkably ameliorated brain injury, reduced apoptotic cell death, maintained mitochondrial functionality and improved cellular parameters, including influx of calcium into cells and the production of reactive oxygen species[176]. These data support the hypothesis that the protective effects of endocannabinoids relies mostly upon their anti-apoptotic and anti-inflammatory effects, opening a new window for their possible use as neuroprotective agents following perinatal asphyxia.

SUMMARY

Cannabinoids emerge as effective neuroprotective compounds, given that the endogenous cannabinoid system is one of the natural mechanisms for controlling damage and induces the healing of diverse injuries. The antioxidant and immunomodulatory properties of cannabinoids, as well as their ability to reduce glutamate release and inducible nitric oxide synthase expression, make these compounds particularly attractive as neuroprotectants in neonatal hypoxic-ischemic encephalopathy given that glutamatergic excitotoxicity, toxic nitric oxide production, oxidative stress, and cytokine release are crucial elements of post-hypoxic-ischemic brain injury in newborns. Moreover, these compounds provide real and exciting prospects for clinical use in the future and give hope that better long-term outcomes may be possible for these patients.

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