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REVIEW ARTICLE

Neuroprotective Effect of Agmatine in Ischemic Vascular Events

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ABSTRACT

Ischemic cerebrovascular diseases are leading cause of mortality and disability worldwide. Given the need for a pharmacological treatment for these diseases, agmatine has gained great interest due to its neuroprotective properties.

This article explores these properties of agmatine in ischemic events and their underlying mechanisms. Agmatine, considered as a neuromodulator, exerts its effects through its interaction with various molecular targets, including glutamate receptors, nitric oxide synthase, and metalloproteinases. Its ability to cross the blood-brain barrier and its role in neurotransmission processes postulate agmatine as a potential candidate for neuroprotection. Agmatine has a positive effect in the central nervous system to counteract excitotoxicity, oxidative stress, inflammation, alteration of the blood-brain barrier and energy disorders during ischemic events. This review describes the multiple interactions of agmatine within the ischemic cascade known to date, showing its ability to mitigate free radical formation, attenuate excitotoxicity, modulate inflammatory responses, stabilize the blood-brain barrier, and preserve mitochondrial function.

These properties position agmatine as a promising therapeutic agent for ischemic cerebrovascular diseases.

Keywords agmatine, neuroprotection, ischemic events, blood-brain barrier, excitotoxicity, oxidative stress, inflammation, mitochondrial function

Abbreviations

CNS Central nervous system
NOS Nitric oxide synthase
CVD Cerebral vascular diseases
CVE Cerebral vascular event
CBF Cerebral blood flow
BBB Blood-brain barrier
ROS Reactive oxygen species

Introduction

Agmatine is an aminoguanidine molecule with a distinct structure when compared to other monoamines, and it is attributed with endogenous neuromodulatory properties[1] (Fig. 1). Agmatine is obtained through the decarboxylation of the peptide L-Arginine (by the action of the enzyme arginine decarboxylase) and is present in foods of animal and plant origin. In addition, the bacteria of the intestinal microbiota produce agmatine in significant concentrations[2]. Exogenous administration of this compound in the form of agmatine sulfate allows rapid absorption of the molecule, which, within minutes, is distributed throughout the body, including the central nervous system (CNS)[3,4]. Agmatine is degraded by the action of the enzyme diamine oxidase, which converts agmatine to guanidino butyraldehyde, as well as by agmatine ureohydrolase, which cleaves the molecule producing urea and putrescine, a precursor molecule of polyamines which is essential for maintaining the viability of neurons[5,6].

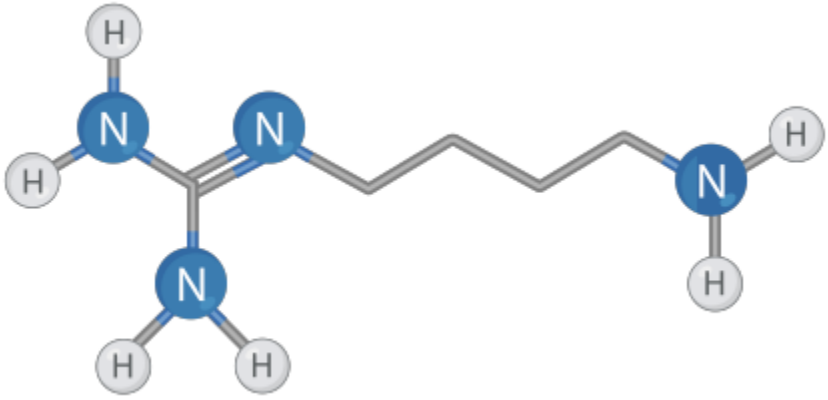

Figure 1. Chemical structure of agmatine

Although agmatine was purified in the early 19th century, its physiological significance and therapeutic potential were neglected for more than 80 years, and it was not until 1994 that it was serendipitously observed that agmatine is able to bind to imidazoline receptors and act as a neuromodulator[7]. Subsequently, it was shown that agmatine modulates glutamatergic neurotransmission through its binding to the N-methyl-D-aspartate (NMDA) glutamate receptor and inactivates the enzyme nitric oxide synthase (NOS)[8,9]. The dual activity of agmatine, as an NMDA antagonist and NOS inhibitor, suggests that it may have a role as an endogenous anti-glutamatergic neuromodulator[10]. In addition, there is recent evidence that exogenous administration of agmatine inhibits the expression of metalloproteinases 2 and 9, proteins responsible for maintaining the integrity of the blood-brain barrier, an indispensable element in the maintenance of brain homeostasis[11,12]. Currently, agmatine is considered to exert modulatory functions, by direct and indirect action, on a broad group of key molecular targets in the regulation of mechanisms considered essential for maintaining health, among which are receptors for neurotransmitters, membrane transporters, nitric oxide synthesis, and polyamine metabolism[3]. In this regard, there is evidence showing that agmatine possesses neuroprotective properties against oxidative stress[13], mitochondrial dysfunction[14], excitotoxicity[15], inflammation[16] and apoptotic response[17]. These effects have been associated with its ability to act on several pharmacological targets, among which are the activation of membrane receptors, including nicotinic, imidazole, alfa2-adrenergic, and 5HT2A and 5HT3 receptors[3,18]. It also inhibits all brain isoforms of the enzyme nitric oxide synthase[8,19] and antagonizes NMDA glutamate receptors[20]. In addition, it was recently reported[21] that agmatine inhibits matrix metalloproteinase 9 in brain endothelial cells. This ability to simultaneously modulate multiple sites makes agmatine a molecule with therapeutic potential in treating complex pathologies, such as ischemic vascular events[22].

AGMATINE IN THE CENTRAL NERVOUS SYSTEM

In the brain, L-Arginine enters through presynaptic terminals into astrocytes where it is decarboxylated and stored in vesicles that are subsequently released by depolarization of Ca++ ion channels[4,23] (Fig. 2), the presence of vesiculated agmatine has also been observed in neurons located in the brain and spinal cord[1].

While it is a cationic molecule at physiological pH, agmatine has been shown to be able to cross the blood-brain barrier in a dose-dependent manner since it is possible to identify an increase in the levels of this molecule in the cerebrospinal fluid after oral or intravenous administration of agmatine[24,25].

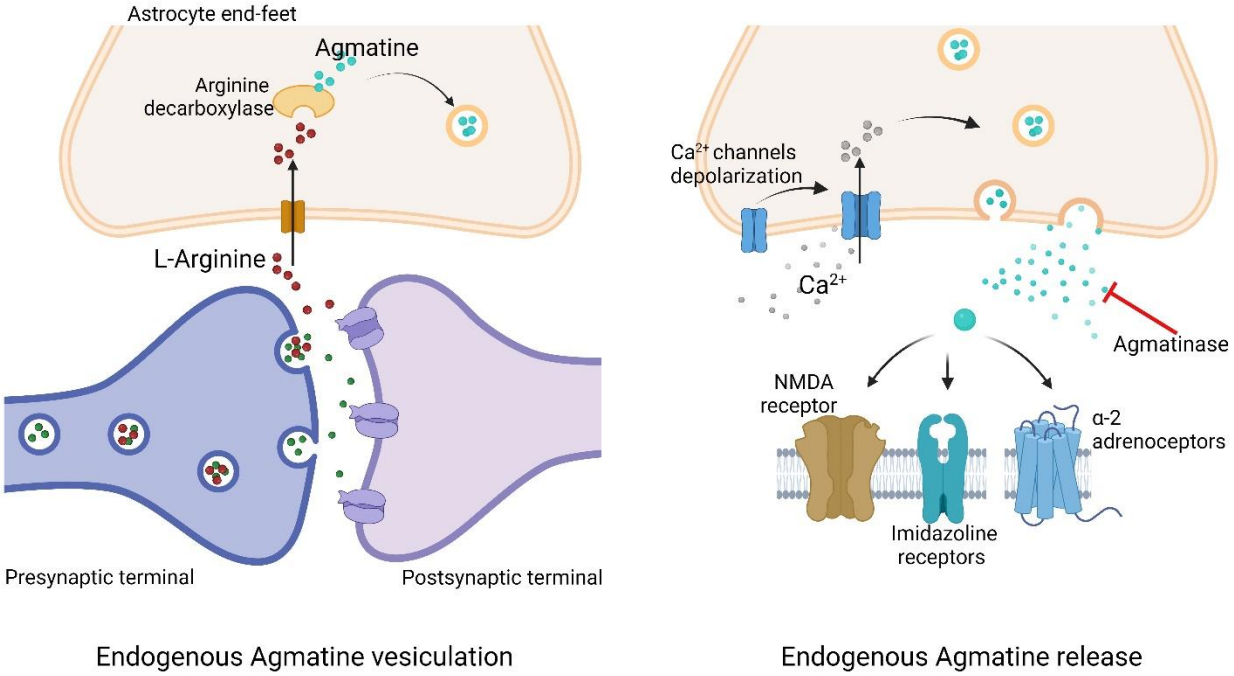

Figure 2. Mechanism of endogenous vesiculation and release of agmatine

The evidence generated in recent years indicates that agmatine could be considered a neurotransmitter due to the presence of various aspects, such as the following: 1. During its synthesis, a unique enzyme participates: arginine decarboxylase[26]; 2. it is stored in specific populations of neuronal cells[27]; 3. It is incorporated into synaptosomes[28]; 4. It is found inside small vesicles located in axon terminals, where classical neurotransmitters such as glutamate and vasopressin are also located[29,30]; 5. Calcium-dependent depolarization releases it from the synaptosome[31]; 6. This molecule has a specific reuptake system[5]; 7. It is inactivated by the action of a specific enzyme: agmatinase[32]; 8. It binds with high affinity to several membrane receptors, such as NMDA, alfa2-adrenergic, and imidazoline receptors[33]; 9. It modulates receptors in the peripheral[34, 35] and central nerves[36]. However, until today, no specific receptor has been identified for agmatine, nor has it been possible to establish whether its effects are presynaptic, postsynaptic, or both, so many authors prefer to consider it a neuromodulator[1].

As mentioned above, among the physiological functions of agmatine is its ability to antagonize NMDA-type glutamatergic receptors[20]. This effect could be related to adaptive mechanisms, such as long-term neuroplasticity since blockade of these receptors induces an increase in neuronal cell proliferation[23]. On the other hand, clinical benefits have been described from the exogenous administration of agmatine during the treatment of CNS conditions, such as neuropathic pain[37] and depression[38]. Furthermore, there is sufficient experimental evidence supporting the potential use of agmatine in the treatment of schizophrenia[39,40]; additionally, it has been observed that there is a correlation between agmatine concentrations in the hippocampus and prefrontal cortex and the degree of learning and memory evocation in laboratory animals[41,42], in addition to the fact that agmatine administration improves the performance of animals in learning and memory paradigms; therefore it is

possible that someday its ability to treat cognitive alterations such as those found in dementia may be evaluated[43]. It is important to note that the neuroprotective effects of agmatine were first described in 1996 and have been confirmed in subsequent studies using various infarct models, including some in which agmatine administration started several hours after the onset of ischemia[44-48]. This neuroprotective effect of agmatine has been related to several mechanisms - described in the following paragraphs - making it a molecule with potential preventive (neuroprotective) and neuronal rescue treatment properties after an ischemic cerebrovascular event.

EFFECT OF AGMATINE ON ISCHEMIC CEREBROVASCULAR EVENTS

Cerebral Vascular Diseases (CVD) are conditions characterized by the sudden onset of neurological alterations that remain for more than 24 hours. These alterations are caused by problems in blood irrigation that may be due to the occlusion or rupture of veins or arteries that administer cerebral blood flow. The World Health Organization (WHO) estimates that about 15 million people worldwide suffer some type of CVE (Cerebral vascular event); of these people, at least 5 million will die, and approximately 5 million will have some type of disability-sensory, motor, or cognitive-after surviving the CVE. The National Heart & Blood Institute (2022) classifies CVEs into two groups: hemorrhagic-present in approximately 15% of cases- and ischemic -approximately 85% of total CVE cases. Ischemic CVEs are characterized by the interruption of cerebral blood flow (CBF) due to the obstruction of cerebral vessels; such obstruction may be caused by a thrombus or an embolus which, by depriving neurons and glial cells of CBF, triggers a series of biochemical processes that influence the development of cerebral ischemia. This set of processes is known as the "ischemic cascade" (Figure 3.

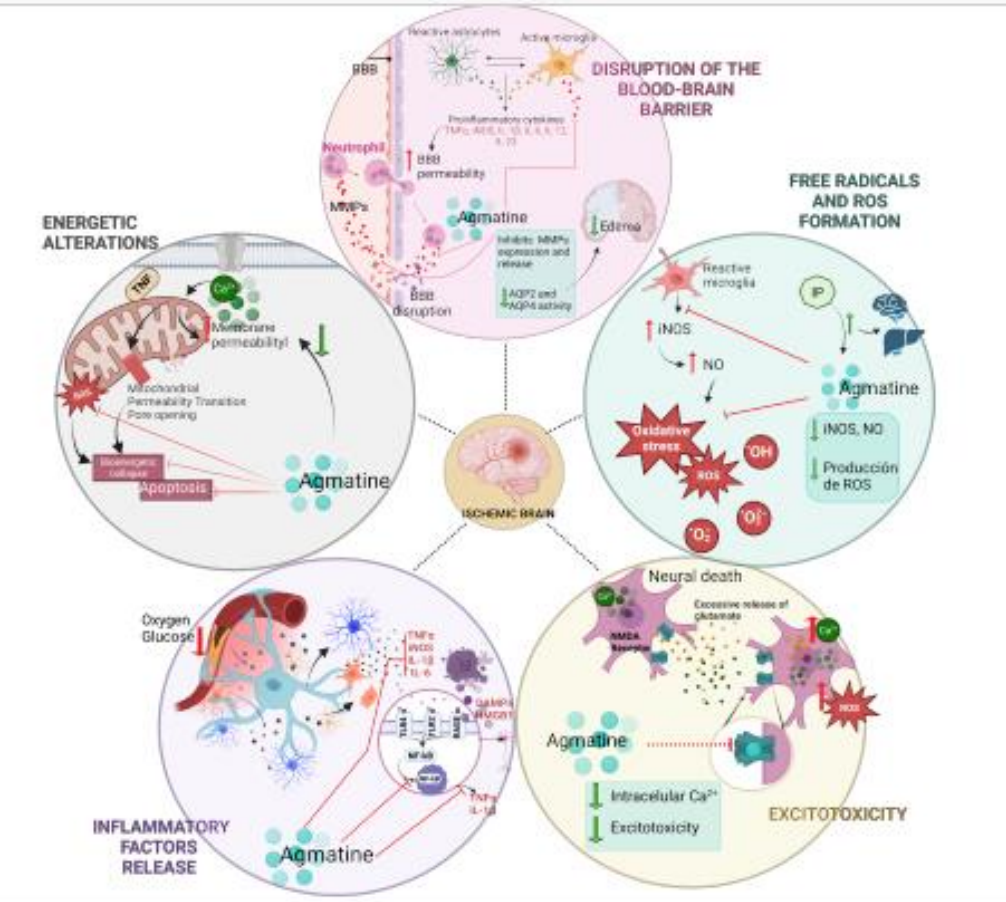

Figure 3. Agmatine interactions in the ischemic brain

Diagram of the ischemic cascade)[49] and includes events such as energetic alterations, formation of free radicals and reactive oxygen species, excitotoxicity, DNA cleavage with release of proinflammatory factors and infiltration of immune cells into the brain parenchyma, among others. Each of these events ultimately contributes to the occurrence of neuronal death[50,51] and the production of enzymes that compromise membrane integrity and cell viability[52,53]. Furthermore, it

should be noted that the ischemic cascade gives way to the formation of an infarct core that is surrounded by a penumbral area, where there are differences in blood flow values. In the nucleus, cell damage is irreversible; however, in the penumbral area, the compromised cells retain metabolic activities that make them susceptible to recovery. Therefore, the penumbra area is considered an important pharmacological target for intervention to prevent the exacerbation of damage caused by the persistence of the above-mentioned pathophysiological mechanisms. Agmatine has been tested in several animal models of cerebral ischemia *in vivo* and *in vitro*, with promising results. In the following, we present evidence of the neuroprotective effect of agmatine and its relationship with the events present during the ischemic cascade.

Interactions of agmatine during the ischemic cascade

1. FORMATION OF FREE RADICALS AND REACTIVE OXYGEN SPECIES

Under hypoxic conditions, such as after an ischemic event, reactive microglia induce an increase in the expression of the enzyme nitric oxide synthase (iNOS), which promotes the synthesis and release of large amounts of nitric oxide (NO), which is responsible for triggering cascades of neuroinflammation and neurodegeneration. Therefore, control of microglia-derived iNOS activity may have a neuroprotective effect in the face of hypoxic injury. In this regard, the work published by Ahn et al. (2011) shows that the administration of agmatine significantly decreases iNOS activity in animals with ischemia due to transient middle cerebral artery occlusion, which attenuates the neuroinflammatory response induced by ischemia[54]. Furthermore, Mun et al. (2010) found that administering agmatine to a global ischemia model increased endothelial NOS (eNOS) expression but decreased iNOS expression, suggesting that agmatine protects the microvasculature in the brain by activation of eNOS and reduces extracellular matrix degradation by inhibition of iNOS and other proteins (e.g., MMMP-9; see below) during the early phase of ischemia[46]. In addition, it has been observed that the occurrence of several transient ischemic events induces the appearance of a phenomenon called ischemic preconditioning (IP), which protects the brain from a lethal ischemic event; for example, it has been described that IP protects hippocampal pyramidal cells from subsequent fatal ischemic events[55]. However, the mechanism behind this phenomenon has not been fully elucidated, so it is interesting to note that after several subsequently induced transient ischemic events, an increase in the synthesis and release of endogenous cerebral and hepatic agmatine has been reported. These findings suggest that IP triggers the agmatine response, which attenuates the damaging effects of ischemia by suppressing iNOS expression and thus may be participating in the IP-induced ischemic tolerance response[56].

2. EXCITOTOXICITY

Glutamate (Glu) is the primary excitatory neurotransmitter of the CNS. This neurotransmitter interacts with several receptors, among which N-methyl-D-aspartate (NMDA), an ionotropic glutamatergic receptor permeable to Ca++, Na+ and K+, stands out. Such receptors are crucial in the CNS as they broadly involve behavioral and cognitive functions[47]. During a hypoxic event, there is an increase in extracellular Glu that overactivates NMDA receptors, thereby increasing intracellular Ca+ and Na+ levels and triggering neuronal excitotoxicity[50,52]. Experimental evidence obtained from hippocampal cells placed in culture shows that agmatine binds to a site located within the NMDA receptor channel pore through the guanidine moiety, resulting in a concentration and voltage-dependent blockade on these receptors. Therefore, the neuroprotective effect of agmatine appears to be due to the decrease in cellular calcium concentrations, which, in turn, reduces Glu-induced damage, cell lysis, and apoptosis[20,57]. Importantly, we failed to detect neuroprotective activity in molecules lacking the guanidine group, as in the case of other endogenous polyamines and the metabolic products of agmatine[58].

3. RELEASE OF INFLAMMATORY FACTORS

Severe inflammation is usually fatal in ischemic patients. This hypoxia-induced inflammatory response is characterized by the activation of microglia and astrocytes, production of inflammatory mediators, and damage-associated molecular patterns (DAMPs) that, in turn, increase the motility of circulating immune system cells. In addition, brain inflammation causes vascular dysfunction that increases BBB permeability[59,60]. One of the first publications about agmatine and its possible effects during the process of neuroinflammation is the work of Lee et al. (2009), where they sought to elucidate the involvement of astrocytes in the neuroprotective effect of post-ischemia agmatine using an *in vitro* model of oxygen and glucose deprivation in a primary culture of astrocytes[61]. Their results indicate that agmatine treatment prevented the death of these cells in culture and induced the translocation of NF-kappaB into the nucleus, suggesting that agmatine may be promoting the regulation of inflammatory

reactions through the activity of NFkappa-B. In contrast, Kim et al. (2016) found that in a model of ischemia with temporal (30 min) middle cerebral artery occlusion, there was a significant reduction of inflammatory factors (IL1-β, and TNF-α), as well as translocation of NFkappa-B factor to the nucleus[62]. The differences in the behavior of NFkappa-B factor in the *in vitro* astrocyte model compared to the intact animal model suggests that this molecule could have different regulatory tasks; on the one hand, it could help prevent astrocytic reactivity and on the other hand, it could prevent exacerbation of damage by delaying or inhibiting the release of inflammatory signaling molecules. The work of Bon-Nyeo Koo's group (2012) identified that administration of agmatine 24 and 72 h after reperfusion improved motor function and behavior in diabetic rats with transient middle cerebral artery occlusion (MCAO)[63]. In addition, immunohistochemical and rt-PCR analyses showed a significant decrease in the expression of inflammatory cytokines, specifically high mobility box 1: RAGE, TLR2, and TLR4. They suggested that the neuroprotective effect of agmatine is related to its ability to modulate ischemia-induced inflammation[62].

Additionally, in a model of intestinal ischemia, Turan et al. (2017) observed that agmatine administration attenuated hypoxia-induced histological damage and decreased the expression of INF-alfa, IL-1beta, and iNOS, thus suggesting that pretreatment with agmatine could decrease reperfusion-induced damage in the small intestine[64]. This effect seems to be due to reduced inflammatory response and oxidative stress. On the other hand, as previously mentioned, agmatine is able to attenuate the activation that hypoxia induces in microglia and astrocytes and, therefore, decreases the synthesis and release of molecules with proinflammatory activity, including NO, TNF-α, and IL-1β[22]. Recently, Lee et al. (2023) published results indicating that agmatine binds with high affinity to interferon regulatory factor 2-binding protein 2 (IRF2BP2), which is actively involved in regulating the inflammatory response. The authors conclude that the neuroprotective effect against neuroinflammation observed after agmatine administration is due to a mechanism involving the expression and release of Kruppel-like factor 4 (KLF4), which induces the M2 phenotype of microglia, largely related to anti-inflammation[65].

4. DISRUPTION OF THE BLOOD-BRAIN BARRIER (BBB)

The blood-brain barrier (BBB) plays a critical physiological role in regulating paracellular permeability, ionic balance, nutrient transport, and cerebral hemodynamics[66]. During and after an ischemic event, impaired BBB promotes injury progression and increases the risk of hemorrhage, generally resulting in poor clinical prognosis and limited medication use[67]. BBB dysfunction, characterized by structural alteration of intracellular tight junctions and increased permeability, is a feature of both ischemic and hemorrhagic cerebral infarcts. It has been observed that approximately 2 hours after the ischemic event, the BBB loses its integrity due to increased activity of proinflammatory cytokines and proteases, among which matrix metalloproteinases 2 and 9 (MMPs) degrade BBB integrity[11]. In this regard, it has been reported that exogenous administration of agmatine in brain endothelial cells with oxygen and glucose deprivation inhibits hypoxia-induced expression of MMP-2 and MMP-9[21]; on the other hand, Jung et al. (2010) demonstrated that the use of retroviruses to induce the expression and release of endogenous agmatine reduces in vitro expression of MMP-2 and MMP-9 in endothelial cells deprived of oxygen and glucose[68]. These results, coupled with those of Ahn et al. (2015) -which, using high-contrast imaging, show that agmatine treatment protects the BBB from ischemia-induced damage in rats- suggest that agmatine may have beneficial effects due to its ability to stabilize the BBB after CVE[11].

On the other hand, cerebral edema is a frequent clinical manifestation in post-ischemic patients. This results from an increase in the volume of water in the brain, and it has a crucial impact on patient morbidity and mortality because it increases intracranial pressure, favors the appearance of herniations, and contributes to the appearance of additional ischemic damage. In a mouse model of transient MAO occlusion, the group of Lee et al. (2009) demonstrated that administration of agmatine significantly decreased the volume of ischemia-induced brain edema, brain water content decreased 24 hours after injury, and functional impairment of the BBB improved[61]. The authors point out that this effect of agmatine may be due to decreased expression of aquaporin 1 (AQP-1)[69]. However, in a paper by Wang et al. (2010), where they also observed a decrease in brain edema after agmatine administration, the authors argue that the effect is due to a decrease in aquaporin 4 (AQP-4) activity[70].

5. EFFECT OF AGMATINE ON EVENTS RELATED TO ENERGETIC ALTERATIONS

The energetic alterations derived from hypoxia-induced mitochondrial compromise are one of the events of the ischemic cascade in which agmatine has been identified as a protective factor. In this sense, it has been reported that this amine is able

to counteract the decrease in energetic capacity that is caused by the mitochondrial permeability transition, a phenomenon dependent on the interaction of Ca2+ with the mitochondrial membrane which consists of the increase in the permeability of the mitochondrial inner membrane to ions and metabolites -with a diameter ≥1. 5kD-, resulting in the production of reactive oxygen species (ROS), which promote transition pore opening, bioenergetic collapse, solute gradient drop, colloid osmotic edema of the mitochondrial matrix, rupture of the outer membrane, and release of proapoptotic factors. Experimental evidence indicates that this protective effect of agmatine can be attributed to the scavenging of ROS, which prevents oxidative stress and, thus, bioenergetic decline[14,71]. Additionally, using an *in vitro* model, Arndt et al. (2009) reported that agmatine protects mitochondrial membrane integrity and protects 5-fluorouracil-induced apoptosis[17]. The authors conclude that the combined effects of agmatine (reduction of oxidative stress, protection of mitochondrial function, and suppression of apoptosis) could be responsible for the beneficial effects that agmatine generated in models of injury and inflammation[17]. These data were subsequently supported by a study performed in an in vitro model of Parkinson's disease. This study's agmatine administration dose-dependently suppressed rotenone-induced cell injury by reducing oxidative stress. In addition, agmatine prevented nuclear translocation of NFkappa-B factor, altered mitochondrial membrane potential, and released pro-apoptotic factors. The authors suggest that agmatine could be used in treating Parkinson's disease due to its ability to protect mitochondrial function and prevent cell death by apoptosis[14].

Conclusions

The impact of acute cerebral ischemia on brain structure and function depends on the severity and duration of the reduction in blood perfusion. When blood flow decreases to 50% of its normal values, there is an increase in lactate production, and water moves from the intracellular to the extracellular space, causing loss of BBB homeostasis and the onset of edema. Additionally, there is a significant increase in the synthesis and release of glutamate, which gives rise to excitotoxicity. The progressive decrease in blood supply affects the generation of the action potential. When the blood flow reaches 20% of its normal value, the neurons lose their ionic gradient, which induces anoxic depolarization, responsible for irreversible neuronal damage[72]. The evidence accumulated over the last few years indicates that there are pathological processes that develop during the hours, and even days, following a CVE; therefore, the mechanisms that induce cerebral ischemic damage are multiple and occur at different times after hypoxia. Energy failure, glutamate-induced excitotoxicity, and depolarization of cells in the infarct zone contribute to the initial damage phase. In contrast, post-ischemic inflammation and apoptosis are responsible for the extent of damage observed in the post-ischemic period. However, it is important to note that the production of reactive oxygen species, including NO, is present at several stages. In the initial phase, NO is produced by arachidonic acid metabolism and nNOS activation. Subsequently, the production of free radicals depends on the infiltration of neutrophils to the infarct zone, while in the late stages, NO is produced by the action of iNOS and COX-2 enzymes[72]. Thrombolysis, commonly used for stroke treatment, is usually effective; however, this approach carries risks, as reperfusion may exacerbate the damage and lead to the rupture of the blood-brain barrier (BBB), ultimately transforming an ischemic event into a hemorrhagic event with further clinical complications[11].

As we have mentioned above, ischemic brain damage depends on multiple mechanisms that intervene at different times after the loss of blood flow. Therefore, rational treatment of brain damage caused by ischemia should be multifaceted in order to target multiple pathogenic sites simultaneously. Therapeutic intervention should be able to restore blood flow, protect the brain from the factors that initiate the ischemic cascade, and minimize the extent of damage associated with the post-ischemic period. The evidence presented in this work indicates that agmatine is a molecule with a potential neuroprotective effect due to its capacity to affect various processes of the ischemic cascade. On the one hand, it is able to reduce the damage, cell lysis, and apoptosis induced by reactive oxygen species and glutamate; in addition, it has a modulating effect on the inflammatory response and protects the integrity of the BBB by reducing the expression of hypoxia-induced metalloproteinases. Agmatine is a molecule that could play a role in this multifaceted therapeutic strategy. As documented in the present work, it has the capacity to modulate several processes within the ischemic cascade, thus preventing neuronal death. Although the evidence may be encouraging, further clinical studies are needed to evaluate its efficacy and determine its safety for humans, which would allow consideration of its use in treating patients with CVE.

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