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Review

Potential Effects of Adropin in Subarachnoid Hemorrhage

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Abstract: Subarachnoid Hemorrhage (SAH) typically, occurs in patients over 55 years of age and can cause a significant loss of productivity. SAH also has a high mortality rate and those who survive often suffer from early and secondary brain injuries that can result from the condition. By gaining a better understanding of the pathophysiology of SAH, it may be possible to identify therapeutic agents to improve outcomes. Adropin is a novel peptide that is primarily secreted in the liver and brain. Research has shown that adropin can activate endothelial NO synthase through post-transcriptional mechanisms. Studies in animal models have demonstrated that therapies using synthetic adropin peptide or adropin overexpression can have positive effects on reducing infarct dimensions and enhancing neurological functioning. In this review, we aim to discuss the potential effect of Adropin on SAH and its potential as a therapeutic agent.

Keywords: Adropin, Subarachnoid Hemorrhage, Intervention, Proposed Mechanisms

Introduction

Adropin is a novel secreted peptide discovered through gene expression analysis in obesity-model mice. The Energy Homeostasis (ENHO) Related gene encodes Adropin (Kumar *et al*., 2008; Aydin, 2014). The central nervous system, heart, pancreas, liver, kidney, coronary artery, and human umbilical vein synthesize adropin (Aydin 2014; Aydin *et al*., 2013). Researchers have found that brain endothelial cells and neurons express adropin in high amounts, while microglia and astrocytes express it at low levels (Yu *et al*., 2017). Compared to other tissues, brain tissues express high levels of the ENHO gene (Zhang *et al*., 2016). The thalamus, midbrain, and hindbrain were observed to express dense echo in several studies. An abundance of Enho mRNA was found in the medial habenula, the hypothalamus, the thalamus, and the medial septal complex. There was high Enho mRNA expression in the substantia nigra compacta, red nucleus, periaqueductal gray, interpeduncular nucleus, and median raphe in the midbrain. As a whole, the findings indicate that the transcript is abundant in areas involved in the control of relaying sensory information (brainstem), particularly temperature regulation (periaqueductal gray), circadian rhythms (lateral geniculate nucleus and medial habenula), cognitive behaviors (thalamus). Postrema and dorsal vagal complex, which regulate ingestive behaviors, also express adropin (Kumar *et al*., 2008). Male brains express ENHO moderately (10%) more than female brains in some areas. There is a peak in ENHO expression in the first decade of life, which subsequently decreases and then remains constant for the following eight decades (Banerjee *et al*., 2021; Yang *et al*., 2018). At the cellular level, ENHO expression is highest among astrocytes and lower in neurons, oligodendrocytes, and endothelial cells (Zhang *et al*., 2020).

The half-life of Adropin is unknown, but it may last up to 30 min, similar to other secretory proteins (Han *et al*., 2019). Adropin is expressed in plasma at a level of 1-10ng/L, with slightly higher levels in males than females (Butler *et al*., 2012).

Lowren and colleagues demonstrated that adropin was important in protecting and regulating endothelial function in vitro conditions (Lovren *et al*., 2010). As a result of adropin treatment, endothelial cells demonstrate more significant proliferation, capillary formation, migration, and elevated endothelial nitric oxide synthase production. The endothelium performs a crucial role in regulating vascular homeostasis, as well as endothelial dysfunction contributes to the development and progress of numerous cardiovascular, infectious, inflammatory, metabolic, and kidney diseases (Verma *et al*., 2023). According to an experimental study higher expression of adropin appears to enhance plaque stability and vascular elasticity to reduce atherosclerosis (Sato *et al*., 2018). Adropin and primary hypertension were negatively correlated in newly diagnosed hypertensive patients, according to (Gu *et al*., 2015; Li *et al*., 2016).

Subarachnoid Hemorrhage

Subarachnoid Hemorrhage (SAH) is a serious medical condition that typically affects patients over 55 years of age and often results in the loss of many years of productivity. In 85% of cases, intracranial aneurysms rupture. While there has been a 17% increase in survival from aneurysmal subarachnoid hemorrhage, survivors often experience cognitive impairments that impact their daily functioning, quality of life, and working capacity (Macdonald and Schweizer, 2017). Early and secondary brain injuries are common following non-traumatic SAH and the pathogenesis of SAH may be affected by early brain injury in the first 72 h of symptom onset (Fujii *et al*., 2013). Secondary brain injury caused by cerebral vasospasm and delayed cerebral ischemia can have a significant impact on outcomes (Eagles and Macdonald, 2019). It is reported that 50-90% of patients with angiography vasospasm experience it (Dorsch and King, 1994), highlighting the need to understand the role of neuroinflammation in the pathophysiology of SAH to identify diagnostic markers and targets for therapeutic intervention (Simon and Grote, 2021). In this review, we discuss the role of Adropin, a novel secreted peptide, in the pathophysiology of SAH, with an emphasis on its role in cerebral vasospasm.

The physiological effects of adropin are caused by the activation of G Protein-coupled Receptor 19 (GPR19) (Stein *et al*., 2016; Thapa *et al*., 2018). Several studies have shown that adropin significantly increases eNOS protein levels and mRNA expression in human umbilical vein Endothelial cells and coronary artery endothelial cells. Nitric Oxide (NO) is produced in the endothelial cell from l-arginine by endothelial NO synthase (eNOS). NO plays several important roles within blood vessels, including anti-inflammatory, antithrombotic, and antiatherosclerotic functions. Moreover, research shows that NO bioavailability plays an important role in insulin sensitivity and metabolic regulation (Baron, 2002; Mather *et al*., 2004; Steinberg and Baron, 2002). There are at least three mechanisms by which eNOS bioavailability is regulated: eNOS transcription, and eNOS synthase through post-transcriptional mechanisms. Results show that adropin triggers the activation of protein kinase Akt, thus resulting in the phosphorylation of the amino acid Ser^{1177}, which is responsible for the posttranscriptional activation of eNOS (Fig. 1). Adropin activates the PI3K-Akt pathway, as shown by its specific use of the PI3K posttranslational activation and reducing reactive oxygen species-mediated NO breakdown. It has been shown that adropin activates endothelial NO inhibitor LY294002.

Moreover, by inhibiting ERK1/2 phosphorylation, PD98059 reduced ERK1/2 phosphorylation induced by adropin. Through these dual pathways, adropin appears to be a new regulator of eNOS. Due to its ability to regulate eNOS production and eNOS-mediated events, adropin enhanced essential Endothelial Cell (EC) functions, including migration, proliferation, on and diminished permeability, and apoptosis through enhanced activation of ERK1/2, eNOS, and Akt.

VEGF activates VEGFR2, a tyrosine kinase receptor that regulates EC survival and function independent of PI3K-Akt and ERK1/2. Several studies have shown that adropin strongly upregulates VEGFR2 in ECs and inhibiting VEGFR2 significantly impaired adropin's effects on eNOS, Akt, and ERK1/2The results suggest that adropin modulates eNOS bioactivity through upstream activation of VEGFR2 and downstream activation of PI3K-Akt and ERK1/2. According to its effects on metabolic control, adropin could be a potential target for vascular disease prevention since atherothrombosis is predominantly a consequence of endothelial dysfunction (Lovren *et al*., 2010).

Nuclear factor erythroid 2-Related Factor-2 (Nrf2) is induced by ERK ½ activation via VEGFR2 and protects neurons from oxidative stress (Kweider *et al*., 2011). DNA repairing ability may be reduced, cell apoptosis might be accelerated and neuron loss might be aggravated if ERK ½ is inhibited (Fig. 2) (Zhao *et al*., 2014). Several studies have demonstrated eNOS expression and endothelial function in whole-body insulin sensitivity (Kumar *et al*., 2008; Baron, 2002; Mather *et al*., 2004; Steinberg and Baron, 2002). The Upregulation of eNOS may improve insulin sensitivity by increasing skeletal muscle blood flow. It has also been shown that various paracrine and neuroendocrine factors regulate metabolism and energy homeostasis by modulating endothelial function and vascular tone (Lovren *et al*., 2010).

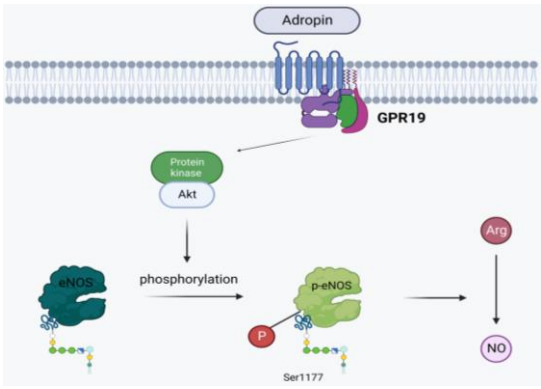

Fig. 1: The physiological effects of adropin are caused by the activation of G Protein-coupled Receptor 19 (GPR19). Activation of protein kinase Akt, thus resulting in the phosphorylation of the amino acid Ser^{1177}, which is responsible for the posttranscriptional activation of eNOS- Nitric Oxide (NO) is produced in the endothelial cell from l-arginine by endothelial NOsynthase (eNOS)

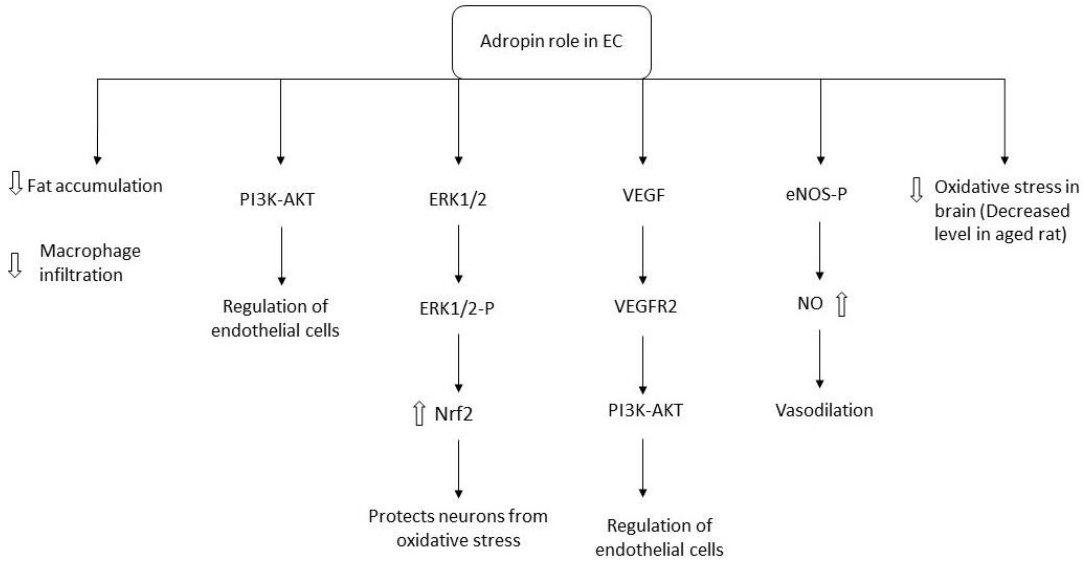

Fig. 2: Adropin effects on endothelial cells

Lipogenic genes and Peroxisome Proliferator-Activated Receptors (PPARs) are regulated byadropin in adipose tissues and the liver, where it all plays a key role in lipogenesis. Furthermore, PPAR-γ was shown to be significantly downregulated in mice with overexpression of adropin (Kumar *et al*., 2008).

In a recent study, adropistimulate the proliferation of 3T3-L1 preadipocytes by regulating ERK1/2 and AKT and inhibits the differentiation of 3T3-L1 cells and rat preadipocytes into mature adipocytes (Jasaszwili *et al*., 2019). Consequently, adropin reduces macrophage infiltration by reducing fat accumulation, as a result of which inflammation is reduced (Zhang *et al*., 2020). The anti-inflammatory properties of adropin have been demonstrated in metabolic disorders, cardiovascular diseases, and other inflammatory diseases. Additionally, adropin levels are associated with inflammation-related genes (particularly Il6 and TNF) in inflammations, tissues, and blood (Gao *et al*., 2016). Ant oxidative stress is one of the effects of adropin. In addition, it reduces oxidative damage to the brain (Yang *et al*., 2018). A reduction in Enho mRNA, protein, and adropin levels was observed in aged rats' brains. Furthermore, phosphorylated and total eNOS levels were markedly downregulated in senior rat brains, which was consistent with significant elevations in $gp91^{phox}$, 4-hydroxynonenal, and oxidative damage markers. There was no significant difference in eNOS protein levels in the prefrontal cortex of aged rats compared with young rats, suggesting that age-related eNOS differences are specific to specific brain structures/regions (Liu *et al*., 2004). Researchers found that aged rats had lower plasma adropin levels than young rats based on these findings. In addition, Enho mRNA and protein levels were significantly reduced in aged rat brains (Yang *et al*., 2018).

A reduction in Akt and caveolin-1 levels were associated with aging, which can modulate eNOS activation (Su, 2015). Similar to humans, mice lose caveolin-1 as they age (Jiang *et al*., 2013). A drop in brain adropin level and decreased Akt, caveolin-1, and eNOS expression in aged rats suggest that adropin could modulate eNOS activity by altering caveolin-1-eNOS interactions (Lovren *et al*., 2010). In stroke, eNOS plays a key role in adropin-mediated neuroprotection (Yang *et al*., 2017a). Additionally, adropin may have effects on blood flow or the blood-brain barrier. There were also direct neurotrophic effects observed on neurons. Currently, it is not known whether adropin can be administered intravenously (Banerjee *et al*., 2021).

Adropin Effects on CNS

Adropin peptide is important in the pathogenesis of several central nervous system disorders, including but not limited to stroke, schizophrenia, Alzheimer's, Parkinson's, bipolar disorder, and Huntington's (Shahjouei *et al*., 2016). Huntington's, Alzheimer's, and Parkinson's disease are neurodegenerative diseases associated with Akt signal defects. Accordingly, adropin may be able to treat neurodegenerative diseases by activating PI3K/Akt (Cinkir *et al*., 2021). Akt is activated by adropin by inducing phosphorylation. The phosphorylation of Akt ensures cell proliferation, differentiation, and survival. The mTOR pathway is also triggered by this path. Apoptosis, neuronal regeneration,

synaptic plasticity, inflammation, and angiogenesis depend on mTOR (Cinkir *et al*., 2021). The study by Wong *et al*. (2014) and colleagues found that adropin is a membrane-anchored protein that binds to NB-3 to promote Notch1 signaling in brain development, which contributes to locomotor activity and coordination (Wong *et al*., 2014). Therefore, a drop in deficient mice displays impaired locomotion and coordination, which defects may cause in the NB-3-mediated Notch1 signaling pathway (Wong *et al*., 2014).

Studies have shown that adropin is detected in cerebellar granular and Purkinje cells (Aydin *et al*., 2013). According to a study, adropin exerts its physiological effect on the hypothalamic paraventricular nucleus by directly affecting nerve excitability (Loewen and Ferguson, 2017). Consequently, adropin successfully restored striatal dopamine levels, making it an attractive therapeutic target for Parkinson's disease and its associated gastric ulcers (Fouda *et al*., 2019). The Paraventricular Nucleus (PVN) was found to respond to 10 nm adropin bath application to three subpopulations of neurons (neuroendocrine, magnocellular, and preautonomic). This suggests that adropin exerts direct, postsynaptic effects on PVN neurons by inhibiting excitatory or inhibitory postsynaptic currents. As a result of these studies, central adropin could exert its physiological actions via interaction with PVN neurons (Loewen and Ferguson, 2017).

Studies have shown that adropin acts directly on neurons at physiological concentrations, enhancing neurite length, thickness, and number in the early stages of cultured neurons. Primary neurites with increased thickness can accommodate more ionic channels and receptors, enhancing synaptic transmission and neuronal conductivity. Primary hippocampal neurons exhibit a significant increase in neuronal activity when exposed to adropin. It is possible that adropin promotes high cognition in humans and animals through its neuritogenic and neuroexcitatory effects on hippocampal neurons. Adropin's effects on vascular performance could be relevant to the aging brain, as dementia associated with aging has been linked to reduced cerebrovascular blood flow (Fouda *et al*., 2019). A change in the cellular environment could also influence aging-related transitions in ENHO gene networks. Cell stress responses could affect transcript levels of ENHO and other transcripts, resulting in age-related inflammation by altering the regulatory framework. It supports the hypothesis that gene networks for ENHO are different in individuals with dementia and those with normal cognitive functions. It appears that increasing expression of adropin improves the cognitive performance of male mice 18 months of age. Adropin treatment can also reverse aging-related cognitive impairment, according to the results from an acute treatment experiment (Banerjee *et al*., 2021).

In the MWM, Y-maze, and OLR tests, adropin improves spatial memory in adult rats. As a result of adropin treatment, improvements in memory appear to be mediated by Akt/CREB/BDNF signaling in the hippocampal area (Ozkan *et al*., 2022).

The GPR19 has been identified as a potential adropin receptor because of its action on the central nervous system. Adropin's inhibitory effect on water deprivation-induced thirst was decreased when GPR19 mRNA levels were reduced in the medial basal hypothalamus of male rats (Stein *et al*., 2016). Under hypoxia, increased expression of adropin results in less water intake, which is controlled by thirst-related nuclei in the brain. Furthermore, adropin inhibits dipsogenic activity in CVOs via TRPV4. Lastly, adropin's anti-dipsogenic effects are mediated by calcium-mediated Camkk-AMPK pathways downstream of TRPV4. As a result of these findings, TRPV4-CamKK-AMPK signaling was involved in the anti-dipsogenic effects of the new small molecule peptide adropin. Pathological rehydration and water sodium balance are better understood in early hypoxia (Yang *et al*., 2017b).

Adropin Potential Effects on Treatment of SAH

Cell-free heme-containing proteins induce endothelial injury in various diseases, including subarachnoid hemorrhage and sepsis. MCP-1 and other chemokines are also responsible for attracting inflammation cells to sites of vascular injury. An adropin-like peptide hormone protects endothelial permeability from hemoglobin-induced endothelial permeability and macrophage migration from MCP-1-induced macrophage migration. Many diseases are characterized by endothelial injury. In a study adropin treatment prevented macrophage infiltration and heme-mediated endothelial injury. Animal models and human tissue specimens need to be investigated further for the effects of adropin therapy (Dodd *et al*., 2021).

Based on the information provided, it is plausible to hypothesize that adropin may play a role in the treatment of Subarachnoid Hemorrhage (SAH) since cell-free heme-containing proteins induce endothelial injury in SAH and adropin has been shown to protect against hemoglobin-induced endothelial permeability and macrophage migration. However, further investigations on animal models and human tissue specimens are necessary to confirm the potential therapeutic effects of adropin in SAH.

Additionally, in another study, mice with collagenase-induced ICH were examined for the role of adropin. The effects of adropin on brain water content and neurological function have been demonstrated. As a result of increased expression of N-cadherin, adropin preserved the function of the BBB by reducing albumin extravasation. The protective effects of adropin were also abolished by the knockdown of Notch1 and Hes1.

Eventually, it has been demonstrated that adropin provides a potential treatment value for ICH by preserving BBB and improving the patient's functional outcome via the Notch1 signaling pathway (Yu *et al*., 2017).

Based on the findings from the study on mice with collagenase-induced ICH, it can be concluded that adropin has the potential as a treatment for SAH. The study demonstrated that adropin preserved the function of the BBB and improved neurological function by reducing albumin extravasation. These effects were mediated by the Notch1 signaling pathway, which suggests that adropin may have similar protective effects in other conditions involving BBB disruption, such as SAH.

It has been shown that exogenous adropin peptide may markedly decrease the size of stroke-induced infarcts in the ischemic mouse brain, which is correlated with an increase of endogenous adropin levels and increased eNOS phosphorylation in the brain (Paul and Candelario-Jalil, 2021). An ischemic stroke preclinical model treated with exogenous adropin peptide or endogenous adropin overexpression showed beneficial results. Significantly, endogenous adropin modulates the brain's susceptibility to ischemia and adropin deficiency worsens stroke outcomes. Efforts should be made to reduce infarct volumes and improve long-term neurological recovery using synthetic adropin in young and old mice. Several studies have shown that adropin treatment reduces stroke-induced BBB damage in the brain of ischemic patients by reducing neutrophil infiltration and oxidative damage. In addition, adropin treatment markedly elevates CBF, which is correlated with elevated phosphorylated eNOS, ERK1/2, and, Akt levels in brain microvessels and increased nitrite levels in plasma (Paul and Candelario-Jalil, 2021). These findings that adropin treatment markedly elevates CBF and increases nitrite levels in plasma may also indicate that it could improve outcomes in SAH by promoting better blood flow to the affected areas of the brain and decreasing vasospasm secondary to SAH.

Stroke mice treated with adropin showed robust improvement in sensorimotor function. Adropin-overexpressing mice were also shown to demonstrate better recognition memory after an ischemic stroke and overexpressing adropin showed protective effects. The results support that adropin therapy or overexpression of adropin may markedly improve cognitive function in old mice (Banerjee *et al*., 2021). Overall, data has suggested that maintaining adropin levels in the brain may be crucial for preventing ischemic brain damage and improving long-term functional outcomes. It has been demonstrated that therapies with synthetic adropin peptide or adropin overexpression have positive effects on reducing infarct dimensions and enhancing neurological functioning both in young and aged mice. ENOS activation is probably responsible for adropin neuroprotective effects (Yang *et al*., 2021). According to these results, adropin inhibits Rho-associated kinase signaling by inhibiting myosin light chain 2 in endothelial cells after hypoxia/low glucose insult. It appears that adropin protects against endothelial dysfunction during ischemic conditions (Yang *et al*., 2016). According to the study results, ischemic preconditioning had neuroprotective effects on middle cerebral artery infarcts correlated with oxidative damage markers and adropin levels (Altintas *et al*., 2016). Based on the given studies, it can be concluded that adropin may have positive effects on SAH, similar to ischemic stroke. Therefore, exploring the potential therapeutic benefits of adropin in SAH patients could be a promising research avenue.

Materials and Methods

A narrative review was carried out to examine the existing literature on the correlation between Adropin peptide and Subarachnoid Hemorrhage (SAH). The search for relevant literature included using keywords such as subarachnoid hemorrhage, aneurysmal subarachnoid hemorrhage, Adropin, and brain aneurysm. PubMed, Embase, and Web of Science databases were comprehensively searched, with articles published prior to December 2022 considered. The search strategy involved combining terms such as subarachnoid hemorrhage, aneurysmal subarachnoid hemorrhage, Adropin, and brain aneurysm. The inclusion criteria for the literature review were as follows: (1) Studies involving preclinical models or human subjects, (2) Studies published in the English language, and (3) Studies investigating the association between Adropin and CNS and SAH.

Conclusion

SAH has a high mortality rate and survivors will suffer cognitive impairments, which impact their daily functioning, quality of life, and working capacity. It is common for early and secondary brain injuries to occur following non-traumatic SAH. Secondary brain injury caused by cerebral vasospasm and delayed cerebral ischemia significantly impact outcomes. Studies have demonstrated that adropin protects endothelial permeability from heme-induced injury and macrophage infiltration, which are common in SAH. Additionally, adropin has been shown to preserve the function of the BBB by reducing albumin extravasation and increasing the expression of N-cadherin, which helps to maintain the integrity of the BBB. Studies have shown that adropin treatment can reduce inflammation and oxidative damage in the brain, as well as elevate cerebral blood flow and improve sensorimotor function. Adropin has also been shown to reduce

infarct dimensions and enhance neurological functioning in preclinical models of ischemic stroke, a condition that can occur as a complication of SAH. Adropin increases NO in the endothelial of the vessel and potentially can reduce vasospasm secondary to the SAH and improve their outcomes. Overall, these findings suggest that adropin may have therapeutic potential in the treatment of SAH by protecting endothelial permeability, preserving BBB function, reducing inflammation and oxidative damage, and improving neurological functioning. Further studies are needed to explore the effectiveness of adropin therapy in SAH and to investigate its potential side effects.

Author's Contributions

Zahra Hasanpour Segherlou: Drafted the article and reviewed it critically for significant intellectual content.

Mohammad Reza Hosseini Siyanaki: Drafted the article and figure preparation.

Brandon Lucke-Wold: Final approval of the version to be submitted.

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