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Endothelial Mitochondrial Dysfunction in Cerebral Amyloid Angiopathy and Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is the most prevalent form of dementia. Cerebrovascular dysfunction is one of the earliest events in the pathogenesis of AD, as well as in vascular and mixed dementias. Cerebral amyloid angiopathy (CAA), the deposition of amyloid around cerebral vessels, is observed in up to 90% of AD patients and in approximately 50% of elderly individuals over 80 years of age. CAA is a strong contributor to vascular dysfunction in AD. CAA-laden brain vessels are characterized by dysfunctional hemodynamics and leaky blood-brain barrier (BBB), contributing to clearance failure and further accumulation of amyloid- β ($A\beta$) in the cerebrovasculature and brain parenchyma. Mitochondrial dysfunction is increasingly recognized as an important early initiator of the pathogenesis of AD and CAA. The objective of this review is to discuss the effects of $A\beta$ on cerebral microvascular cell function, focusing on its impact on endothelial mitochondria. After introducing CAA and its etiology and genetic risk factors, we describe the pathological relationship between cerebrovascular amyloidosis and brain microvascular endothelial cell dysfunction, critically analyzing its roles in disease progression, hypoperfusion, and BBB integrity. Then, we focus on discussing the effect of $A\beta$ challenge on endothelial mitochondrial dysfunction pathways, and their contribution to the progression of neurovascular dysfunction in AD and dementia. Finally, we report potential pharmacological and non-pharmacological mitochondria-targeted therapeutic strategies which may help prevent or delay cerebrovascular failure.

Keywords

Alzheimer's disease; amyloid; apoptosis; blood-brain barrier; cerebral amyloid angiopathy; endothelial cells; mitochondria; neurodegeneration; reactive nitrogen species; reactive oxygen species

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INTRODUCTION

Dementia affects more than 40 million people in the world, and its incidence is expected to triple by 2050 [1]. Alzheimer's disease (AD) is the most common form of dementia, although AD is known as a multifactorial disorder with multiple etiologies and different pathological manifestations. Mixed pathology dementias account for more than half of dementia cases, with amyloidosis and vascular disease being the most frequent combination [2–9]. The hallmark pathological characteristics of AD are senile or neuritic plaques of insoluble amyloid- β (A β) and neurofibrillary tangles (NFTs) of phosphorylated microtubule-associated protein tau (P-tau) [10]. In addition to these well-known lesions, pathological changes in the brain vasculature strongly contribute to clinical AD, as well as to vascular and mixed dementias [2, 11].

One of the most common but often overlooked pathological features of AD is cerebral amyloid angiopathy (CAA), the deposition of insoluble A β in cortical and leptomeningeal arteries, arterioles, and around the capillary walls [12, 13]. CAA is present in up to 90% of AD patients [14] and frequent in cognitively normal older individuals, at a prevalence of up to 50% after 80 years of age [15–17]. The perivascular accumulation of A β in CAA leads to cerebral endothelial cell (CEC) dysfunction and death [18]. Microvascular degeneration induced by CAA consequently results in hypoperfusion, neuroinflammation, blood-brain barrier (BBB) dysfunction, and oxidative stress, further contributing to the neurodegenerative process [18] and conferring substantial risk of vascular lesions in AD and other dementias [19]. This vascular viewpoint of the pathogenesis of AD and dementia is being investigated with increasing interest [2, 8, 20–22].

Cerebral endothelial cells control the chemical milieu of the brain by regulating molecular transport across the BBB and modulating cerebral blood flow (CBF). Vascular mechanisms of AD pathophysiology that are recognized among the Vascular Contributions to Cognitive Impairment and Dementia [8, 23] include chronic cerebral hypoperfusion, macro- and micro-hemorrhages, CEC dysfunction and death, capillary loss, neurovascular damage and impaired clearance [2, 8, 24–29]. The effects of altered cerebral hemodynamics on cognitive function have been extensively reviewed [11, 30–33], and small vessel damage and BBB breakdown are important mechanisms in AD pathogenesis that can contribute to the accumulation of A β and altered homeostasis in the brain [15, 34].

The objective of this review is to discuss the effects of A β on CEC dysfunction, specifically focusing on the pathways by which A β challenge affects the endothelial mitochondria. After introducing the clinical and neuropathological features, risk factors, and genetics of AD, this review describes the pathological effects of CAA on the cerebrovasculature such as hypoperfusion, ischemia, and BBB damage. Next, we describe the putative mechanisms responsible for amyloid-induced mitochondrial dysfunction in CECs, and analyze the specific components of such CEC mitochondrial dysfunction, encompassing energy metabolism, production of reactive oxygen (ROS), nitric oxide (NO) and reactive nitrogen species (RNS), calcium (Ca²⁺) crosstalk between the endoplasmic reticulum (ER) and mitochondria, and apoptosis. The review concludes with a discussion of therapeutic

strategies against cerebral endothelial mitochondrial dysfunction and recommendations for future directions of relevant studies.

Alzheimer's disease

Overview—AD is a progressive neurodegenerative disease in which gradual memory and cognitive impairments are typically preceded by extracellular deposition of A β as diffuse and insoluble plaques in the brain [35]. A β plaque deposition is followed by intracellular accumulations of NFTs and neurodegeneration [36]. A β is a product of β -secretase (BACE) and γ -secretase-catalyzed proteolysis of the amyloid- β protein precursor (A β PP), a single-pass transmembrane protein in the plasma membrane [10]. The A β plaques in AD brains are primarily comprised of A β ₄₂, but A β ₄₀ represents the main component of vascular amyloid deposits in CAA [12, 37]. Recent literature that utilized imaging and biomarker data from the large-scale database Alzheimer's Disease Neuroimaging Initiative (ADNI) has reported that brain vascular dysfunction precedes both A β and tau pathology, and cognitive impairment in AD [38]. Preclinical studies also argue for the importance of vascular, rather than brain parenchymal amyloid, in inducing early cognitive impairment in mice [11, 22, 39, 40].

Risk factors and genetics—Aging is the most common and widely known risk factor for developing AD [41]. Other risk factors for AD include the female sex for increased risk of onset but male sex for duration of survival after clinical diagnosis [42], low educational and occupational status, low mental status, low mental and physical activity [43], and cardiovascular risk factors such as hypercholesterolemia, hypertension, cerebrovascular diseases [44], hyperhomocysteinemia [45], smoking, obesity [46], and diabetes mellitus [47]. Traumatic brain injury (TBI) has also recently been recognized as a possible risk factor for AD [48] and associated with cerebrovascular dysfunction [49], but the exact link between TBI and AD still remains under debate [50].

The ϵ 4 allele of the apolipoprotein E (*APOE*) gene is the most common genetic risk factor [51] for AD. Sporadic AD, or late-onset AD, which occurs in older patients who are over the age of 65 years [52], accounts for 95–99% of all AD cases [52, 53]. Familial AD is referred to as early-onset AD, occurring in patients younger than 65 years of age [52]. Familial AD involves mutations in the presenilin-1 (*PS1*), presenilin-2 (*PS2*), and *APP* genes [54]. Main mutations on A β which lead to familial AD include A2V (A2V), English (H6R), Tottori (D7N), K16N (K16N), Flemish (A21G), Osaka (E22), Italian (E22K), Dutch (E22Q), Arctic (E22G), Iowa (D23N), Piedmont (L34V), Swedish (KM670/671NL), and Indiana (V717F) [55–57]. Most familial forms of AD are marked by increased kinetics of A β aggregation [55], which results in early age of onset. Multiple mutations in the A β sequence and other aggregation-prone proteins such as ABri and Adan are particularly associated to early-onset aggressive forms of CAA and related to dementia with cerebral hemorrhage [18, 58].

Cerebral amyloid angiopathy

In addition to the extracellular accumulation of A β plaques from frontal and temporal lobes and the hippocampus and limbic systems to other regions of the brain parenchyma, a

hallmark amyloid pathology of AD is the deposition of A β in the walls of cortical and leptomeningeal arteries, arterioles and capillaries, termed CAA [12]. Emerging evidence suggests that there are two types of CAA: arterial and capillary/pericapillary CAA. In arterial CAA, insoluble A β deposits in the vessels' tunica media and adventitia along the perivascular space [12, 59, 60]. Pericapillary CAA is suggested to be marked by insoluble A β aggregates along the glia limitans surrounding the capillary and may indicate the initial stages of A β accumulation due to impaired glymphatic clearance of A β and other waste products [13]. Capillary CAA is the deposition of A β within the capillary wall (i.e., between the endothelium and basement membrane) [13].

CAA is one of the most common cerebral small vessel diseases and the most frequent cause of lobar intracerebral macro- and micro-hemorrhages in elderly individuals [15, 37, 61]. CAA is also a major risk factor for white matter hyperintensities observed via magnetic resonance imaging (MRI), hypoperfusion and ischemia, BBB damage in cerebral arterioles and capillaries, and molecular dysfunctions of CECs [37]. Therefore, CAA confers substantial vascular contribution to the pathogenesis of AD [8, 19]. Hereditary CAA is most commonly caused by Dutch, Italian, Arctic, Iowa, Flemish, and Piedmont mutations in the *APP* gene, as well as the Icelandic mutation in the *CST3* gene (L68Q), and the British and Danish mutations in the *ITM2B* gene [62]. CAA has been recognized as a cause for intracerebral hemorrhage, brain hypoperfusion, ischemia and BBB dysfunction, and all forms of cerebrovascular disease discussed below [22, 63].

Hypoperfusion and ischemia

A prolonged reduction of CBF, or hypoperfusion, due to hypotension, cardiovascular disease, or CAA, limits glucose and oxygen delivery and impairs brain function. An increasing amount of evidence has demonstrated that cerebrovascular dysfunction may precede and exacerbate amyloid accumulation, linking cardiovascular risk factors to the development of CAA and AD [64, 65]. Hypoperfusion seems to accelerate the development of CAA, which, in turn, is associated with the development of cortical microinfarctions [66] and reduced CBF, establishing a degenerative feed-forward process for the brain's neurovascular unit. Indeed, cerebrovascular hypoperfusion could be one of the underlying inducers for the development of AD, as drugs that treat hypoperfusion are able to alleviate some of the AD symptoms [67]. However, the cause-effect relationship between CAA and hypoperfusion is still unclear [68]. It appears that hypoperfusion could result in vascular damage that leads to A β accumulation which, in turn, leads to more vascular damage. For example, accumulating evidence supports the role of A β in mediating vasoconstriction through the reduction of NO bioavailability as a direct consequence of increased ROS levels (discussed in the NO section below).

In support of the concept that cerebral hypoperfusion precedes amyloid accumulation, in the APP Swedish and Indiana mutation mice (APP-SweInd), cerebral hypoperfusion was shown to accelerate A β deposition [69]. Actually, cerebral hypoperfusion alone resulted in a decrease in learning and memory, and increased A β_{40} and A β_{42} levels in the hippocampus [70]. Similar results were obtained in transgenic Swedish, Dutch and Iowa, mutation (Tg-SwDI) mice, where cerebral hypoperfusion increased A β_{40} and A β_{42} in the cerebral

vasculature and increased microinfarctions [71]. It was further demonstrated that APP/PS1 mice with carotid occlusion shifted A β toward high molecular weight oligomeric species [72], suggesting an increase in amyloid burden and disease progression. Additionally, hypertension accelerated the development of cognitive deficits, A β deposition in the microvasculature, and BBB dysfunction in the Tg-SwDI mice [65].

The increase in A β_{40} /A β_{42} levels may be due to increased activity of the amyloidogenic pathway through the upregulation of BACE and γ -secretase [70]. Hypoxia inducible factor (HIF), a transcription factor that is activated due to hypoxia, was shown to induce BACE-1 expression [73], therefore possibly contributing to the hypoperfusion-induced A β accumulation. In an *in vitro* model of cerebral hypoperfusion using oxygen and glucose deprivation, brain capillary endothelial cells upregulated the expression of HIF-1, BACE1, A β PP, and A β_{42} levels [74]. However, other studies have portrayed a possible neuroprotective role of HIF [75, 76]. It is possible that the activation of HIF-1 could mediate protective mechanisms during acute hypoxic conditions to prevent cell death but that its prolonged activation could lead to BACE-1 activation, and eventually cell death [77].

The blood-brain barrier in CAA and AD

The BBB is formed by tight junction proteins that connect CECs, which are surrounded by the basement membrane and astrocytic end feet, thereby creating a selectively permeable barrier [78]. Cerebral endothelial cells strictly regulate the transport of solutes between the lumen of blood vessels and the interstitium of the brain parenchyma, and, consequently, regulate brain homeostasis [79]. As a result, the BBB is necessary to prevent the entry of toxins, waste products, or other detrimental agents into the brain. Dysfunction of CECs can result in a disrupted, or “leaky,” BBB, which allows the influx of toxic and undesirable molecules into the brain, impairing brain homeostasis, and inducing inflammation and aberrant neurovascular function. It is hypothesized that BBB dysfunction leads to neuronal damage and A β accumulation in the brain [79]. This may occur progressively throughout the neurovascular unit, comprised of CECs, pericytes, smooth muscle cells, astrocytes, microglia, oligodendroglia, and neurons [80]. For example, hypoperfusion and A β accumulation can lead to P-tau and NFT accumulation and injury in neurons and activate microglia [80].

Substantial loss of endothelium is observed in human AD brains [81]. Along with decreased mitochondrial content in CECs [82], brain biopsies [82], and MRI of AD patients [83] have shown BBB dysfunction and leakage. Transgenic murine models of CAA and AD have further corroborated such BBB dysfunction [84]. Furthermore, in humans with AD pathology (CSF A β_{42} positive), cerebrovascular capillary and BBB damage was evident. Interestingly, they also reported that BBB damage was present in humans with early cognitive dysfunction (CSF A β_{42} negative), demonstrating that BBB damage is present before A β accumulation and at the onset of mild cognitive dysfunction, therefore making it capable to serve as a predictor of cognitive function [21]. These data suggest that BBB dysfunction may be an early mediator of AD pathology.

BBB leakage or dysfunction is primarily mediated by the loss of tight junction (TJ) proteins. Cerebrovascular endothelial TJs include integral membrane proteins such as occludin,

claudins, and junctional adhesion molecules (JAMs). These, in turn, interact with intracellular scaffold proteins such as zonula occludens and cytoskeletal proteins. Surprisingly, only claudins, and not occludin and JAMs, appear to be essential in BBB integrity (reviewed in [85]). Given the importance of BBB in brain homeostasis, it is of no surprise that BBB dysfunction observed in CAA and AD pathology may be mediated by the loss of TJs. An increase in BBB permeability coupled to a reduction of TJ proteins claudin-1 and claudin-5 was observed in isolated rat brain microvessels treated with human A β ₄₀ and in microvessels from transgenic mice expressing hAPP with the Swedish mutation (Tg2576) [86]. Furthermore, claudin-5 expression, and to a lesser extent occludin, were downregulated in multiple areas of human postmortem AD brains [87]. Interestingly, the authors also demonstrated that there is an inverse correlation between cortical claudin-5 and occludin expression, and insoluble A β ₄₀ and CAA. Overall, BBB dysfunction contributes to the development of CAA and AD pathology through the downregulation of TJ proteins, where claudin-5 appears to be the main player.

Energy metabolism of endothelial mitochondria

As mitochondria represent the energy source for our brain cells, mitochondrial damage is likely to be one of the earliest events in the development of AD [88–95]. Mitochondrial dysfunction can not only induce energetic failure in the AD brain, but also contribute to the activation of executioner caspases, such as caspase 3, thereby mediating neuronal, vascular and glial cell death, and the progression of AD pathology [96–100].

Physiological metabolism—Compared to peripheral endothelial cells, CECs are much more dependent on mitochondrial respiration for their energy metabolism and survival. Mitochondrial volume in rat brain capillary CECs was 8–11% of the total cytoplasmic volume, or 2–4 times the mitochondrial volume of peripheral endothelial cells [101]. Their higher susceptibility to apoptosis and disruptions in TJs due to hypoxic or ischemic conditions also suggest their dependence on mitochondrial metabolism [102]. Glucose is the main obligatory metabolic substrate of the human adult brain and its CECs [103]. A 55-kDa isoform of glucose transporter 1 (GLUT1) imports glucose into the CECs [104]. Glucose then undergoes glycolysis, and subsequently lactate fermentation or mitochondrial metabolism, or the pentose-phosphate pathway [103]. The adenosine triphosphate (ATP) turnover rate and the percentage of ATP produced by the mitochondria relative to overall CEC metabolism are not yet clear.

Effects of A β on mitochondrial metabolism—In CECs, it is not yet known the mechanisms by which A β disrupts mitochondrial metabolic pathways and systems such as the tricarboxylic acid (TCA) cycle, electron transport chain (ETC), and oxidative phosphorylation, nor the enzymes aberrantly affected by A β . However, due to the conserved structure-function of enzymes in many of these metabolic pathways, it is possible that A β may affect these metabolic processes and ATP production in CECs in similar ways as in non-synaptic brain, neuronal, and astrocytic mitochondria.

Exposure to A β _{25–35}, which is the shortest fragment capable of forming β -sheet fibrils and retaining the toxicity of the full length A β _{40/42} peptides [105], led to decreased ATP

production by 42% in neurons and by 28% in astrocytes compared to respective control cells [106]. In non-synaptic brain mitochondria, 30-min exposure to A β _{25–35} inhibited pyruvate dehydrogenase and α -ketoglutarate dehydrogenase activity, consequently downregulating pyruvate production and TCA cycle activity [107]. In cerebellar granule neurons, exposure to A β ₄₂ impaired ETCs nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase (Complex I) and cytochrome c (CytC) oxidase (Complex IV) activities [108] and bound to Complex IV in human neuroblastoma cells [109]. Another metabolic damage caused by amyloid proteins is the impairment of oxidative phosphorylation. Both A β PP and A β ₄₀ bound to subunit α of F₁F₀-ATP synthase and partially inhibited ATP production in neuroblastoma cells [110]. However, immortalized microvascular CECs challenged with the aggressive vasculotropic variant A β ₄₀-E22Q, did not show a decrease in mitochondrial ATP production [111].

In this study, amyloid peptides exerted differential effects on SH-SY5Y (neuronal) and CECs, inducing a slight decrease in ATP levels in neuronal cells and a substantial increase in CECs. A possible explanation is that these cell types handle mitochondrial dysfunction by differentially resorting to aerobic glycolysis [112]. Additional studies in primary CECs are needed to clarify whether this is a characteristic linked to the immortalized nature of the human CEC used (hCMEC/D3), or if different pathways characterize the neuronal/astrocytic versus endothelial mitochondrial metabolic effects of A β .

ENDOTHELIAL PRODUCTION OF REACTIVE OXYGEN SPECIES

Metabolism and ROS production in the mitochondria are interconnected. In the ETC, Complex I and coenzyme Q (CoQ)-CytC reductase (Complex III) are primarily responsible for producing superoxide anions (O₂^{•−}) [113]. Complex I produces O₂^{•−} in the process of oxidizing NADH into NAD and pumping a proton from the mitochondrial matrix into the intermembrane space [113]. Complex III produces O₂^{•−} as it oxidizes CoQ to reduce CytC and pump a proton into the intermembrane space [113]. Molecules of O₂^{•−} are potent oxidants and, therefore, need to be reduced and “detoxified.” In the mitochondrial matrix, superoxide dismutase (SOD) 2 reduces O₂^{•−} into H₂O₂, a less toxic ROS [114]. Then, glutathione peroxidase (GPX) catalyzes the reduction of H₂O₂ into H₂O through the oxidation of reduced glutathione (GSH) into its oxidized form (GSSG) [114]. Catalase in the mitochondrial matrix can also convert H₂O₂ into water and molecular oxygen [114]. Glutathione biosynthesis is catalyzed by glutathione reductase using the oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) into NADP and is crucial in antioxidant activities in the mitochondria [114]. In the intermembrane space, copper and zinc-containing SOD1 reduces O₂^{•−} into H₂O₂, and GPX reduces H₂O₂ into H₂O [114].

Damage to the ETC and oxidative phosphorylation due to A β in CEC mitochondria can lead to excessive production of mitochondrial ROS, oxidative stress, further damage to the mitochondria and cell, and exacerbation of vascular amyloid pathology. Changes in mitochondria-specific redox homeostasis due to A β have been investigated in transgenic CAA mouse models [115, 116]. Proteomic analysis of CECs isolated from cerebral arteries from the Circle of Willis and its main branches in the double transgenic APP-SweInd mice showed upregulated expressions of mitochondrial copper-zinc SOD1 and CytC oxidase

subunit 6C [115]. A similar double transgenic CAA mouse model of Tg2576 that also expresses PS1 (L166P) showed that there was upregulated SOD2 expression and O_2^- production in CECs of leptomeningeal arteries [116]. The mitochondria-derived ROS, in turn, exacerbated CAA pathology in such mice [116], suggesting a downward spiral of A β accumulation and oxidative stress in the cerebrovasculature.

Recent studies by our group also clearly demonstrated an increase in H_2O_2 production by mitochondria isolated after treatment of human CEC with A β peptides [111], accompanied by the loss of mitochondrial membrane potential (ψ) [111, 117], both prevented in presence of carbonic anhydrase inhibitors (CAIs). However, the mechanism responsible for the increase in H_2O_2 induced by A β is not fully understood. While other studies have investigated the effect of exogenous A β on the oxidative stress and ROS production of CECs, not necessarily specifically to the mitochondria, it is likely that the redox responses in CECs include mitochondrial ROS production and antioxidant enzymes. In bEnd3 mouse CEC line and primary rat CECs, A β_{25-35} exposure led to increased cellular ROS production and downregulated cellular SOD activity [118, 119], as well as increased cellular lipid peroxidation, decreased GSH/GSSG, and downregulated cellular catalase, glutathione-S-transferase, and GPX activities [119]. Interestingly, exposure to lower concentrations of A β may allow CECs to recover from mitochondrial and overall cellular oxidative stress. Rat CECs (RBE4) exposed to low dosage of A β_{40} had increased cellular ROS levels and decreased GSH/GSSG ratio at 3, 6, and 12 hours, which normalized by 24 hours [120]. Glutathione reductase expression also increased at 12 and 24 hours, which also suggests an antioxidant recovery effect in CECs [120].

NITRIC OXIDE AND REACTIVE NITROGEN SPECIES

The vasoactive substance NO is essential for the regulation of CBF and maintenance of cerebral function. Proper regulation of vascular tone and CBF is essential to couple brain metabolic demand with supply. Nitric oxide is synthesized by the nitric oxide synthases (NOS) and promotes vascular relaxation through its actions on vascular smooth muscle cells. There are three main NOS isoforms: neuronal NOS (nNOS) and endothelial NOS (eNOS), which are activated by Ca^{2+} -calmodulin, and Ca^{2+} -independent inflammation-induced inducible NOS (iNOS). Various studies have proposed the existence of a mitochondrial localized NOS (mtNOS) [121, 122], but these findings have been challenged (discussed in detail in [123, 124]).

Given the importance of CBF in the development and progression of CAA and neurodegeneration [125], the role of NOS in the cerebrovasculature has been widely studied. A β_{1-40} has been shown to induce vasoconstriction in cerebral vascular endothelial cells *in vitro* and *in vivo* [126]. The expression of capillary eNOS was found to negatively correlate with the amount of NFTs and senile plaques in postmortem human brains [127]. In addition, mice lacking eNOS (eNOS $^{-/-}$) displayed increased levels of A β PP and BACE1 in whole brain tissue [128]. Indeed, partial deficiency of eNOS (eNOS $^{-/+}$) is enough to induce vascular changes that result in CAA, BBB breakdown, and cognitive deficits [129]. Therefore, a growing number of studies demonstrate a crucial role of eNOS for proper

endothelial function and a potential link between decreased eNOS expression and activity and the development of CAA.

Interestingly, PC12 cells with the double Swedish mutation (APP^{Sw}; K670M/N671L) have increased NO levels possibly due to increased NOS activity [130]. This increase in NO levels may be mediated by iNOS, which was found to be elevated in AD postmortem brains [127]. Although enhanced NO production seems to be a beneficial, rather than detrimental effect, NO availability is severely impaired in the presence of ROS, specifically O_2^- , due to the spontaneous formation of peroxynitrite ($ONOO^-$), which has oxidizing and nitrating capabilities. This is further supported by data demonstrating that administration of a ROS scavenger to Tg-SwDI mice enhanced vascular function [40], indicating that ROS may decrease NO bioavailability, thus contributing to vascular dysfunction and vasoconstriction in CAA.

The spontaneous formation of $ONOO^-$ occurs faster than the O_2^- dismutation by MnSOD [131], suggesting that even during normal MnSOD activity and expression, the formation of $ONOO^-$ is evident. Most importantly, NO inhibits SOD by preventing O_2^- binding, therefore increasing intracellular ROS and possibly RNS [132]. Interestingly, phosphorylation of eNOS at serine-1177 increases O_2^- promotion [133], an event that is observed in the brain of the Tg2576 mouse model of amyloidosis [134]. Furthermore, nitrotyrosine content, an indicator of $ONOO^-$ damage, was increased in brains from AD patients when compared to controls [135, 136]. Importantly, in Tg2576 mice, an increase in 3-nitrotyrosine levels and amyloid deposition were observed in cerebrovascular endothelial cells [137]. In cultured cortical neurons, treatment with a NOS inhibitor was able to reduce reactive species after $A\beta_{22-35}$ treatment suggesting that $ONOO^-$ is the major reactive species formed in these conditions [138]. The excessive $ONOO^-$ formation severely impairs NO bioavailability leading to vasoconstriction and reduced CBF. In a mouse model of AD, endothelial-NO bioavailability was severely impaired [139], which could be secondary to excessive $ONOO^-$ formation. This data is further supported by studies in human umbilical vein endothelial cells where $A\beta_{25-35}$ decreased NO levels [140].

In ratCECs, mitochondrial membrane depolarization induced NO production through a Ca^{2+} -mediated activation of eNOS [141]. Elevated NO inhibits cellular respiration in vascular endothelial cells by inhibiting Complex IV [142, 143] and is associated with mitochondrial dysfunction in PC12 cells with the $A\beta_{PPSw}$ K670M/N671L mutation [130]. Membrane depolarization, with elevated ROS production, in particular O_2^- , will then induce production of $ONOO^-$, depleting the NO pool. Therefore, it appears that $A\beta$ deposition in the cerebrovasculature is accompanied by an increase in $ONOO^-$ damage and consequently a reduction of NO bioavailability resulting in vasoconstriction and reduction of CBF.

The formation of nitrotyrosine has also been observed to influence $A\beta$ aggregation. Nitrosylation of $A\beta$ peptide induced formation of $A\beta$ oligomers [144] which are known to be the most toxic aggregation species [145] for multiple brain cell types including CECs and smooth muscle cells [146–148]. Other groups have reported that nitrotyrosine promotes $A\beta$ aggregation and plaque formation [149]. Although the data available is still controversial, it suggests a possible role of RNS, most specifically $ONOO^-$, in the regulation of $A\beta$ toxicity

and plaque formation. On the other hand, coadministration of ONOO^- with $\text{A}\beta$ in rat CECs, did not exacerbate vascular cytotoxicity [150]. Overall, the data suggests that NO and RNS could potentiate endothelial mitochondrial dysfunction in AD. This can not only cause cerebrovascular dysfunction due to the absence of NO, but also DNA damage due to the reactive properties of ONOO^- [151].

CALCIUM, ENDOPLASMIC RETICULUM, AND MITOCHONDRIAL PERMEABILITY TRANSITION PORE

The endoplasmic reticulum (ER) is primarily involved in the regulation of Ca^{2+} homeostasis. Under physiological conditions, the mitochondria can uptake Ca^{2+} after it has been released from the ER to increase metabolic activity through the activation of several enzymes [152]. In pathological conditions leading to sustained activation of ER stress, caspase activation and CytC release is activated through the mitochondria [153]. Although these mechanisms have been extensively studied in neuronal cells [154, 155], evidence suggest that similar mechanisms may be involved in $\text{A}\beta$ -induced brain endothelial dysfunction.

Rat brain endothelial cells treated with $\text{A}\beta_{40}$, the primary $\text{A}\beta$ isoform in CAA, displayed increased ER stress response markers and disrupted Ca^{2+} homeostasis as observed by a decrease in ER Ca^{2+} and an increase in cytosolic and mitochondrial Ca^{2+} [120, 156]. Both, $\text{A}\beta$ and ER Ca^{2+} depletion induce the formation of a transient receptor potential (TRP) channel in the plasma membrane provoking an increase in Ca^{2+} conductance, which further leads to an increase in BBB permeability and endothelial dysfunction [157, 158]. However, whether the effects of $\text{A}\beta$ on ER Ca^{2+} depletion are mediated through the TRP channel or through other mechanisms is still unclear.

The mitochondria are key mediators of cell death. Under conditions of high matrix Ca^{2+} , elevated ROS production, and loss of ψ , mitochondrial permeability transition (mPT) can be induced. Pathological mPT leads to the formation of an irreversible, non-selective pore termed the mPT pore (mPTP) resulting in mitochondrial swelling, outer membrane rupture, and the induction of cell death. ER calcium depletion, loss of ψ , and CytC release observed in $\text{A}\beta_{40}$ treated rat, murine and bovine endothelial cells [156, 159] may be indicative of mPTP formation. Further supporting this data, Yin and collaborators demonstrated that inhibition of the mPTP using Cyclosporin A (CsA) was able to prevent $\text{A}\beta$ -induced release of the mitochondrial apoptotic protein second mitochondria-derived activator of caspases (Smac) [105]. In primary cultures of rat astrocytes and hippocampal neurons, a reduction of mitochondrial inorganic phosphate prevented $\text{A}\beta$ -induced mitochondrial depolarization and cell death, possibly through inhibition of Ca^{2+} -induced mPTP [160]. However, in human CECs treated with the aggressive vasculotropic mutant $\text{A}\beta_{40}\text{-E22Q}$, both mitochondrial and cytoplasmic Ca^{2+} , measured by Fluo-4 and Rhod-2 fluorescence, respectively, were significantly decreased [111]. Taken together, the data suggests that $\text{A}\beta$ may disrupt Ca^{2+} homeostasis, leading to an increase in BBB permeability and mitochondria-mediated cell death through the release of pro-apoptotic factors, although further research is needed to clarify the underlying mechanisms.

MITOCHONDRIA-MEDIATED APOPTOSIS IN CEREBRAL ENDOTHELIAL CELLS

Physiology of intrinsic apoptosis

Intrinsic apoptosis can be activated by aberrant metabolic activity in pathways such as oxidative phosphorylation [161], overproduction of ROS and RNS [161], DNA damage [162], ER stress [163], and ER release of excess Ca^{2+} into the mitochondria [163]. Aberrant intracellular cascade signaling and DNA-binding proteins can also induce intrinsic apoptosis by inhibiting anti-apoptotic kinases that promote cell growth such as protein kinase B (Akt) [164] or activating pro-apoptotic kinases such as apoptosis signal-regulating kinase 1 (ASK1) [165, 166] and c-Jun N-terminal kinase [167].

These intracellular triggers activate Bcl-2-associated X (Bax) and Bcl-2 homologous antagonist killer (Bak) channels [168], which allow the translocation of CytC released from oxidized cardiolipin (also released by mPTP) [169, 170] and the pro-apoptotic signaling protein Smac, also known as direct inhibitor of apoptosis protein (IAP) binding protein with low pI homolog (Diablo) [168]. Translocated CytC joins apoptotic protease activating factor 1 and procaspase-9 to form the apoptosome [171]. The activated apoptosome activates caspase-3 and -7, which subsequently catalyze substrate proteolysis, and ultimately cell death [171].

A β -induced cerebral endothelial intrinsic apoptosis

A β has been shown to directly activate mitochondria-mediated apoptotic pathways as well as key signal transduction kinases that have downstream pro-apoptotic mitochondrial targets in CECs [146]. Bovine brain microvascular endothelial cells can uptake human A β_{40} , likely through ATP-dependent endocytosis [147, 172], suggesting that internalized A β_{40} may induce intrinsic apoptosis through ER stress, ROS/RNS production, and intracellular Ca^{2+} overload.

This concept is supported by the A β -induced early ER stress in RBE4 cells, shown by increased expressions of cyclic AMP-dependent transcription factor 4 (ATF4) and 6a (ATF6a) [156], Grp78, and xbp-1 [156, 173], and consequent early and prolonged depletion of ER Ca^{2+} and increase in cytosolic Ca^{2+} [120, 156]. These events were followed by upregulation of caspase-9, caspase-3, CytC release, and loss of ψ [156]. Alongside ER stress, the intracellular accumulation of ROS such as O_2^- and toxic molecules such as 4-hydroxynoneal caused by A β_{40} exposure may lead to intrinsic apoptosis, measured by loss of ψ [174]. Another indication of intrinsic apoptosis is damage to the mitochondrial DNA, measured by reductions in long-chain PCR products. A β_{25-35} was shown to induce mitochondrial DNA damage after 24 and 48 hours in murine CECs [159], which was accompanied by CytC release, caspase-3 and -8 activation. Accordingly, vasculotropic A β_{40} mutants (such as the Dutch, Piedmont, and Iowa mutants), as well as wild-type (WT)-A β_{40} , all triggered CytC release and caspase activation pathways [117, 146, 147]. Mitochondrial apoptotic pathways may also be engaged as a secondary step during extrinsic apoptotic cascades. Importantly, the pro-apoptotic effects induced by A β peptides in cerebrovascular cells, as well as in neuronal and glial cells, have been mainly attributed to oligomers and

protofibrils [117, 146, 147, 175, 176], which are typically accumulated extracellularly and acting as alternative receptor ligands.

Physiology of extrinsic apoptosis

Extracellular death ligands, such as FasL and tumor necrosis factor (TNF) α , bind to the extracellular domains of transmembrane death receptors (DRs) to trigger extrinsic apoptosis [177]. Common death receptors include the cluster of differentiation 95 (CD95 or Fas), TNF receptors (TNF-R), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) DRs [178–180]. The ligand-DR binding allows the DR's cytosolic domain to recruit adaptor proteins such as the Fas-associated death domain protein (FADD) [179]. These adaptor proteins then recruit multiple procaspase-8 molecules, which then undergo auto-cleavage and activation [177]. Caspase-8 cleaves and activates BH3 interacting-domain death agonist (Bid) into truncated Bid (tBid), which activates the Bax/Bak proteins, while also cleaving procaspases-3 and -7 into their active caspase forms for substrate proteolysis and cell death [171, 180].

Effect of A β on extrinsic endothelial apoptosis

These pathways have been investigated in A β -exposed endothelial cells using primary murine, rat, and human CECs, mouse (bEnd.3), rat (REB4), and human (hCMEC/D3) brain endothelial cell lines, and CECs derived from A β PPsw mice. In hCMEC/D3 cells, A β ₄₀-E22Q oligomers have been shown to directly bind to TRAIL DR4 and DR5, leading to increased caspase-8 activity, downregulation of cellular FADD-like IL-1 β -converting enzyme-inhibitory protein (c-FLIP, an inhibitor of caspase-8), consequent Bid cleavage and CytC release [148]. Translocation of Bax from the cytosol to mitochondria has also been previously shown [181] in human CECs. Interestingly, A β may also trigger an extrinsic-like apoptotic mechanism through the activation of CEC surface receptors other than apoptotic death receptors, such as the receptor for advanced glycation endproducts (RAGE) [182] and scavenger receptor cluster of differentiation 36 (CD36) [183].

A transmembrane multi-ligand receptor that's part of the immunoglobulin (Ig) superfamily, RAGE's physiological function involves inflammatory and immune responses [184]. Previous studies have shown that RAGE directly binds to A β ₄₀ and A β ₄₂ [185] and may be involved in their apical-to-basolateral transport in CECs [186]. In bEnd.3 cells, A β _{1–42} (WT) activated RAGE and subsequently induced ER stress, marked by ER stress markers 78-kDa glucose-regulated protein (Grp78), X-box binding protein 1 (xbp-1), and CCAAT-enhancer-binding protein homologous protein [182]. This ER stress subsequently led to both mitochondria-dependent and -independent apoptosis. Mitochondria-dependent apoptosis was shown through an increased ratio of Bax/Bcl-2 expression, and mitochondria-independent apoptosis was seen by an increased expression of caspase-12. Adiponectin has been recently shown to decrease RAGE expression and attenuate apoptosis in A β -challenged bEnd3 cells. However, binding of A β oligomers to RAGE has been recently debated [187].

CD36 is a multi-ligand, class B transmembrane glycoprotein receptor that is involved in fatty acid transport [188] and negative regulation of angiogenesis in microvascular endothelial cells [189]. In Tg2576 mice, the binding of A β ₄₀ to CD36 was found necessary

for NADPH oxidase-mediated ROS production and oxidative stress in cerebral endothelial cells of penetrating arterioles [183].

Signal transduction kinases

In addition to the extrinsic, extrinsic-like, and intrinsic apoptotic triggers, kinases and phosphatases involved in metabolic signal transduction pathways were shown to activate mitochondrial apoptotic pathways after primary murine CECs' exposure to A β peptides. A β_{25-35} activated protein phosphatase 2A (PP2A), a serine/threonine phosphatase with a diverse range of signaling substrates, in primary mouse CECs [190]. It is suggested that PP2A may dephosphorylate and activate ASK1. In this study, ASK1 was shown to activate the MAPK kinase 3 and 6 (MKK3/6) pathways, which activated the p38/MAPK pathway. This led to the activation of p53, which activates Bax and inhibits Bcl-2, and ultimately mitochondria-mediated apoptosis.

Moreover, primary murine CECs' exposure to A β_{25-35} was shown to downregulate active, phosphorylated forms of Akt and upregulate Bcl-2-associated death promoter (Bad) [191] and Bcl-2-like protein 11 (Bim) [192], which are downstream inhibition targets of Akt. Bad translocated from the cytosol to mitochondria, and inhibited Bcl-XL [191]. The downregulation of Bcl-XL and upregulation of Bim led to release of mitochondrial Smac, which was mediated by the mPTP, eventually allowing activation of caspases and apoptosis to proceed.

Effect of different amyloid peptides

The extent of activation and upregulation of apoptotic pathways involving the mitochondria may vary depending on the A β peptide, its rate of oligomerization, and the CEC type. In murine and rat cell lines as well as primary mouse CEC cultures, WT A β_{40} , A β_{25-35} , and A β_{42} induced mitochondria-mediated apoptosis, upregulation of apoptotic proteins, and mitochondrial DNA damage as described in above subsections. However, by WT A β_{40} amyloid exposure in human CECs showed more delayed effects when compared to vasculotropic mutants associated with CAA and cerebral hemorrhage such as the Dutch (E22Q), Piedmont (L34V), and Iowa (D23N) A β_{40} mutants [117, 146–148, 181].

The speed of amyloid oligomerization matched the speed and extent of apoptosis in hCMEC/D3 cell: A β_{40} -E22Q immediately oligomerized and was faster compared to A β_{40} -L34V and A β_{40} (WT) [146–148]. Similarly, aggregation kinetics and apoptosis induction were fastest and greatest for A β_{40} -D23N (isoD), then A β_{40} -D23N, and then A β_{40} (isoD) [117].

This suggests that mutated amyloid peptides more quickly induce mitochondria-mediated apoptosis in CECs than WT peptides, especially A β_{40} , which has slower aggregation kinetics compared to A β_{42} . Ultimately, all A β_{40} peptides (E22Q, L34V, and WT) induced the activation of caspase-8, which was followed by caspase-9 activation and apoptosis [148]. However, the different kinetics of mitochondrial dysfunction and apoptosis induction clearly reflect the earlier age of disease onset in people possessing these vasculotropic mutations and highlight the relevance of these studies and of mitochondrial dysfunction mechanisms in differentiating between different disease pathologies.

Primary human CEC cultures showed similar results with A β ₄₀-E22Q but not A β ₄₂ [181]. While A β ₄₂ was shown to have the highest tendency to form fibrils, more so than A β ₄₀-E22Q, only A β ₄₀-E22Q, and neither A β ₄₀ (WT) nor A β ₄₂ (WT), increased apoptosis and triggered cytosolic Bax translocation to and colocalization with the mitochondria and CytC release after 12 and 24 h of exposure [181]. It is possible that this lack of upregulated mitochondrial apoptotic activity may be due to the presence of fibrils, instead of oligomers, in A β ₄₂ preparations, and to the delayed effect shown by A β ₄₀ (for which apoptosis and CytC release were only present after 3 day challenge in hCMEC/D3 cells) [117, 146, 148].

CONTRIBUTION OF MITOCHONDRIAL FUNCTION IN NEUROGENESIS

Neurogenesis, or the formation of neurons from neuronal progenitor cells, has been observed in the hippocampus during adulthood and has been proposed as a mechanism for learning and memory formation [193, 194]. Mitochondria have also been shown to play a role in neurogenesis [195, 196]. Therapies that enhance mitochondria bioenergetics such as daily oxaloacetate (OAA) treatment [197, 198], high-intensity exercise [199], and tauroursodeoxycholic acid [200] improve neurogenesis. On the other hand, selective knockout of apoptosis inducing factor during embryonic development induces mitochondrial dysfunction and impairs neurogenesis and cognitive function [201]. Although the mitochondria appear to have an important function in neurogenesis, the role of neurogenesis in AD and cognition, learning, and memory warrants further investigation [202]. For a detailed discussion on the contribution of mitochondrial function in neurogenesis during AD, the reader is directed elsewhere [203–205].

THERAPEUTIC STRATEGIES

Mitochondria-targeted therapies

Recognizing that mitochondria are the main sources of ROS, many mitochondrial-targeted therapies have focused on the development of antioxidant molecules that accumulate in the mitochondrial matrix. One of such molecules is the Szeto-Schiller peptide (SS-31), also known as Bendavia or MPT-131, which accumulates in the mitochondria in a ψ -independent manner, inhibits mPTP, and serves as a ROS scavenger [206]. The peptide SS-31 was found to prevent age-associated neurovascular uncoupling and mitochondrial dysfunction in microvascular CECs, and this in turn prevented the age-related cognitive decline in WT mice [207]. Although the SS-31 peptide seems to target some of the main features observed in CAA, its potential beneficial effects in cerebral amyloidosis have not been studied.

Another mitochondrial-targeted antioxidant is MitoQ, a ψ -dependent triphenylphosphonium (TPP⁺)-ubiquinone derivative that was shown to prevent H₂O₂-induced apoptosis [208]. This compound was recently approved to undergo clinical trials as a dietary supplement for AD patients where the endpoint is to assess its effects on CBF and carotid endothelial function (). This clinical trial emerged from the observation that mitoQ could reverse vascular defects due to aging, possibly in an NO-dependent manner [209]. It is possible that decreasing ROS levels increases bioavailability of NO by preventing ONOO⁻ formation and restoring vascular function. In addition, in a triple transgenic AD mouse

model (3×Tg-AD) of APP with the Swedish mutation, MAPT P301L, and PSEN1 M146V, a five-month treatment with mitoQ prevented cognitive decline, nitrotyrosine formation (a marker of ONOO⁻ damage), synaptic dysfunction, and caspase 3/7 activation [138]. However, other studies have reported adverse effects of MitoQ in the kidney, indicating that MitoQ induced mitochondrial swelling and membrane depolarization due to the increased inner mitochondrial membrane permeability [210]. The effects of MitoQ in reducing ONOO⁻ damage could be especially beneficial in CECs, were it appears that lack of NO bioavailability and excessive ROS production enhance or contribute to the progression of AD.

During the last few years, an FDA-approved drug, the CAI methazolamide, was shown to protect CECs, smooth muscle cells, neurons and glial cells against Aβ-mediated mitochondrial dysfunction and apoptosis [117, 146, 211]. These promising results led our group to also evaluate the effects of the analog CAI acetazolamide on mitochondrial function in cerebral vascular cells as well as neuronal cells, and the mechanisms responsible for the protective effects [111]. Both CAIs were able to prevent H₂O₂ production, loss of ψ , activation of caspase 9, and CytC release; thereby inhibiting apoptosis [111]. Due to their FDA-approved use in several medical conditions including glaucoma and high altitude sickness, as well as the potential added benefits in improving CBF and vasoreactivity [212, 213], these compounds could provide a multi-target novel pharmacotherapy for CAA and AD with potential protective effects against cerebrovascular mitochondrial dysfunction.

Multiple studies have focused on the modulation of mitochondrial bioenergetics to prevent cognitive dysfunction. It was reported that the inhibition of Complex I with a small molecule, CP2, is able to prevent the cognitive decline and Aβ accumulation in APP, PS1, and APP/PS1 transgenic mice [214, 215]. Other studies have demonstrated that OAA, can modulate mitochondrial bioenergetics in mice and the human cell line SH-SY5Y [197, 198], reducing neuroinflammation and enhancing the expression of genes involved in neurogenesis [197]. OAA supplementation appears to be a promising therapy since it is well tolerated in humans [216]. These findings further support the mitochondrial as a possible player in neurodegeneration and provide evidence that the regulation of mitochondrial bioenergetics could be developed as a possible therapeutic approach to AD and CAA.

Although less common, some studies have evaluated the use of natural compounds that appear to target the mitochondria directly. One of such compounds is *Centella asiatica*, an aquatic plant. Water extracts from *centella asiatica* were able to improve learning and spatial memory in Tg2576 mice (open field test, Morris water maze) without affecting Aβ levels [217]. In contrast to these results, in a different model of AD, PS/APP mice expressing Swedish APP and M146L presenilin 1, a decrease in hippocampal Aβ₄₀ and Aβ₄₂ levels was observed after *centella asiatica* treatment for 8 months starting at 2 months of age (before the development of amyloid burden) [218]. The effects of *centella asiatica* might be mediated by a reduction of ROS [218] and preservation of mitochondrial function [219]. However, the actions of *centella asiatica* on brain microvascular endothelial cells during amyloidosis have not been studied.

Clinical trials and non-pharmacological therapies

Currently, there are no effective cures for AD or CAA. The National Library of Medicine only displays a total of 12 registered clinical trials for CAA that have been or are currently underway (<https://clinicaltrials.gov/ct2/results?cond=Cerebral+Amyloid+Angiopathy&term=&cntry=&state=&city=&dist=>). However, only three (or 25%) involve a pharmacotherapeutic approach; the vast majority intend to understand the relationships between several risk factors and CAA through imaging studies. Of the three clinical trials involving pharmacotherapy, two phase II clinical trials have been completed which involved an immunotherapy treatment with Ponezumab, which was demonstrated to be inefficient [220, 221], and a glycosaminoglycan mimetic [222] for which the results have not yet been posted.

Interestingly, a recently approved clinical trial will examine the potential effect of oleocanthal rich-extra virgin olive oil (EVOO) on preserving cognitive function and preventing the BBB deficits in mild-cognitive impaired patients. The Mediterranean diet, which is high in EVOO, has been associated to a reduced risk of AD and cognitive impairment [223, 224]. To further explore the potential effects of EVOO against cognitive decline, various *in vivo* and *in vitro* studies have been performed. In wild type mice brain endothelial cells, EVOO increased the expression of proteins involved in A β ₄₀ clearance and this was associated with enhanced clearance of radioactively labeled ¹²⁵I-A β ₄₀ after acute injection [225]. Although the 2013 study used WT animals, the same group later demonstrated that in Tg-SwDI mice, treatment with oleocanthal decreased total A β accumulation in the hippocampus and brain microvessels; these findings were further confirmed in a human microvascular endothelial cell line [226]. In both studies they demonstrate that the effects of oleocanthal is on A β clearance since it does not affect A β production. If effective, it would provide a non-pharmacological option to the prevention of cognitive decline and AD. EVOO, known for its antioxidant effects, could also have potential beneficial actions against mitochondrial dysfunction in CAA and AD [227, 228].

Various natural compounds have been associated to beneficial effects in cognitive decline. One of these compounds is *Crocus sativus*, also known as saffron, a spice that is retrieved from a flower. A phase II clinical trial was held in Iran (Iranian Clinical Trials Registry IRCT138711051556) where 30 mg/kg of saffron a day was able to confer the same beneficial effects of donepezil, but with a marked reduction of the side effects, after 22 days of treatment in mild and moderate cognitive impaired individuals [229]. Crocus supplementation for 12 months improved the Mini-Mental State Examination scores as well as the latency of P300, measurements of cognitive function [230]. Although the clinical results are mild but positive, saffron appears to exert its effects by improving A β clearance through upregulation of BBB transporters and prevention of astrocyte activation in 5XFAD mice [231]. However, these studies did not evaluate if these effects improved cognitive function. Lastly, a possible role for physical activity on human cognitive function has been observed. Acute exercise improved pre-frontal but not hippocampal cognitive function [232], while long-term exercise attenuated hippocampal volume decrease and improved memory functions [233]. The beneficial effects resulting from increased physical activity in AD occur at different cellular and metabolic levels, mitochondria being preferential target

organelles [234–236]. Moreover, high intensity exercise was shown to increase VEGF-A expression, which induces proliferation and migration of vascular endothelial cells [199].

FUTURE DIRECTIONS

Brain endothelial cells are responsible for regulating BBB function, a process in which mitochondria play an essential role. This is further supported by data demonstrating that brain endothelial cell mitochondria occupy from 8 to 11% of cytoplasmic volume in contrast to non-cerebral endothelium in which mitochondria occupy less than 6% of cytoplasmic volume [101]. Cerebral amyloid angiopathy is characterized by endothelial dysfunction, disruption of the BBB, and vascular damage; all which appear to precede cognitive dysfunction in AD. Therefore, understanding the mechanism by which endothelial dysfunction occurs and the role of the mitochondria in this pathology could provide new therapeutic strategies to prevent AD. However, the role of the mitochondria in endothelial dysfunction during CAA is understudied and further research is needed in multiple directions, among which understanding the mechanisms by which A β affects mitochondrial metabolism, respiration, Ca²⁺ signaling, as well as initiation of mitochondria-mediated apoptotic pathways. Clarifying these pathways will support the development of novel biomarkers and therapeutic approaches for CAA, AD, and vascular mixed dementias.

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Fig. 1.

Nitric oxide and amyloid in vascular dysfunction. Endothelial NO is primarily responsible for mediating vasodilation through its actions on vascular smooth muscle cells. NO is synthesized by Ca²⁺-dependent eNOS and nNOS, and inflammation-induced iNOS. Both, inflammation and the increase in cytosolic Ca²⁺ increase NO production. Amyloid induces vascular dysfunction by promoting vasoconstriction through a direct action on vascular cells or through its effects on mitochondrial function. Amyloid also induces endothelial mitochondrial dysfunction, which results in excessive production of superoxide and a disruption of Ca²⁺ homeostasis. Mitochondria-produced superoxide spontaneously reacts with NO to form reactive peroxynitrite, reducing the NO pool and further contributing to vasoconstriction.

Fig. 2.

Amyloid-induced apoptotic pathways involving the mitochondria in cerebral endothelial cells. Various Aβ peptides have been shown to induce mitochondria-mediated extrinsic and intrinsic apoptosis in CECs. This figure depicts the effects of Aβ on membrane receptors, such as RAGE, CD36, and DR4/5, which activate both mitochondria-dependent and independent caspases (Cas) and apoptosis. Both Aβ₄₀ and Aβ₄₂ increase mitochondrial and cytosolic ROS, and mitochondrial CytC release. Aβ peptides can also activate PP2A-mediated cascade signaling and inhibit Akt, leading to the activation of pro-apoptotic proteins in the cytosol and mitochondria. Aβ also induces mitochondrial DNA damage that facilitates CEC apoptosis.