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Di Marco, L.Y., Venneri, A., Farkas, E. et al. (3 more authors) (2015) Vascular dysfunction in the pathogenesis of Alzheimer's disease - A review of endothelium-mediated mechanisms and ensuing vicious circles. Neurobiology of Disease, 82. 593 - 606. ISSN 0969-9961

https://doi.org/10.1016/j.nbd.2015.08.014

Vascular dysfunction in the pathogenesis of Alzheimer's disease – A review of endothelium-mediated mechanisms and ensuing vicious circles

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Highlights

- Age and cardiovascular conditions initiate cerebral hypoperfusion
- Chronic cerebral hypoperfusion (CCH) causes blood-brain barrier (BBB) dysfunction
- BBB dysfunction causes oxidative stress, inflammation, and mitochondrial dysfunction
- BBB dysfunction mediates a vicious circle causing CCH aggravation and neuronal damage
- BBB dysfunction is worsened by Aβ-endothelium interaction

Summary

Abbreviations

ABC	=	ATP binding cassette
AD	=	Alzheimer's Disease
ApoE	=	Apolipoprotein E
AQP	=	Aquaporin
Aβ	=	Amyloid-β
AβPP	=	Aβ Precursor Protein
BACE-1	=	β-site AβPP Cleaving Enzyme 1
BBB	=	Blood-Brain Barrier
CBF	=	Cerebral blood flow
CSF	=	Cerebrospinal fluid
eNOS	=	Endothelial Nitric Oxide Synthase
ER	=	Endoplasmic Reticulum
ET-1	=	Endothelin 1
GLUT-1	=	Glucose Transporter 1
HIF-1α	=	Hypoxia-Inducible Factor 1α
ICAM-1	=	Intercellular adhesion molecule-1
IFN-γ	=	Interferon-γ
IL	=	Interleukin
iNOS	=	Inducible nitric oxide synthase
IP_3	=	Inositol 1,4,5-triphosphate
LRP-1	=	Low-density lipoprotein Receptor-related Protein 1
MEOX-2	=	Mesenchyme Homeobox gene-2
MCP-1	=	Monocyte Chemoattractant Protein-1
mitoK_{ATP}	=	ATP-sensitive potassium channels
mtDNA	=	Mitochondrial DNA
NF-kB	=	Nuclear Factor kappa B
NFT	=	Neurofibrillary Tangle
NO	=	Nitric Oxide
PECAM-1	=	Platelet Endothelial Cell Adhesion Molecule 1
PET	=	Positron Emission Tomography
PKC	=	Protein kinase C
P-gp	=	P-glycoprotein
RAGE	=	Receptor for Advanced Glycation End-products
RNS	=	Reactive Nitrogen Species
ROS	=	Reactive Oxygen Species
TEER	=	Trans-endothelial electrical resistance
TGF-β	=	Transforming Growth Factor-β
TNF-α	=	Tumour Necrosis Factor-α
TJ	=	Tight Junction
UPR	=	Unfolded Protein Response
VEGF	=	Vascular Endothelial Growth Factor

Abstract

Late-onset dementia is a major health concern in the aging population. Alzheimer's disease (AD) accounts for the largest proportion (65-70%) of dementia cases in the older population.

Despite considerable research effort, the pathogenesis of late-onset AD remains unclear. Substantial evidence suggests that the neurodegenerative process is initiated by chronic cerebral hypoperfusion (CCH) caused by aging and cardiovascular conditions. CCH causes reduced oxygen, glucose and other nutrient supply to the brain, with direct damage not only to parenchymal cells, but also to the blood-brain barrier (BBB), a key mediator of cerebral homeostasis. BBB dysfunction mediates the indirect neurotoxic effects of CCH by promoting oxidative stress, inflammation, paracellular permeability, and dysregulation of nitric oxide, a key regulator of regional blood flow. As such, BBB dysfunction mediates a vicious circle in which cerebral perfusion is reduced further and the neurodegenerative process is accelerated. Endothelial interaction with pericytes and astrocytes could also play a role in the process. Reciprocal interactions between vascular dysfunction and neurodegeneration could further contribute to the development of the disease.

A comprehensive overview of the complex scenario of interacting endothelium-mediated processes is currently lacking, and could prospectively contribute to the identification of adequate therapeutic interventions.

This study reviews the current literature of in vitro and ex vivo studies on endothelium-mediated mechanisms underlying vascular dysfunction in AD pathogenesis, with the aim of presenting a comprehensive overview of the complex network of causative relationships. Particular emphasis is given to vicious circles which can accelerate the process of neurovascular degeneration.

Keywords: Alzheimer's disease; blood-brain barrier permeability; cerebral hypoperfusion; endothelial dysfunction; mitochondrial dysfunction; ultrastructural damage

1. Introduction

Late-onset dementia – an irreversible and debilitating condition characterized by progressive cognitive decline – is a major health concern in the aging population. Alzheimer's disease (AD) accounts for the largest proportion (65-70%) of dementia cases in the older population [1–3]. Despite considerable research effort, the pathogenesis of sporadic AD remains unclear. A longstanding hypothesis proposed by Hardy and Higgins [4] – known as the "amyloid cascade hypothesis" – suggests that AD pathology is initiated by the deposition of insoluble amyloid β (Aβ) fragments resulting from amyloid precursor protein (AβPP) proteolysis. An imbalance between Aβ production and clearance results in toxic Aβ concentrations, neuronal tau protein hyperphosphorylation and consequent neurofibrillary tangle formation [5]. However, substantial evidence [6–10] suggests that the neurodegenerative process is initiated by chronic cerebral hypoperfusion caused by aging, vascular conditions such as hypertension, atherosclerosis, type II diabetes, hypercholesterolemia [6, 8–11], and possibly cardiac conditions such as atrial fibrillation and chronic heart failure [7, 12, 13]. Chronic hypoperfusion causes reduced oxygen, glucose and other nutrient supply to the brain [14], with direct damage not only to parenchymal cells, but also to the blood-brain barrier (BBB), a key mediator of cerebral homeostasis. BBB dysfunction mediates the indirect neurotoxic effects of chronic hypoperfusion by promoting oxidative stress [6, 15, 16], inflammation [17–20], impaired glucose transport across the BBB [21–23], BBB permeability [21, 24, 25], and dysregulation of nitric oxide (NO) [26–28], a key mediator of vascular tone and regional blood flow regulation [29, 30]. As such, BBB dysfunction could mediate a vicious circle in which cerebral perfusion is reduced further and the neurodegenerative process is accelerated [31–33]. Adjacent to endothelial cells, astrocytes and pericytes could also play a role in this process. Indeed, astrocytes are known to amplify the endothelial response during inflammation [34, 35], and some studies suggest that pericytes could contribute to microvascular tone regulation and regional blood flow distribution [36–38]. Reciprocal interactions between vascular dysfunction and neurodegeneration have also been proposed [39, 40], supported by a large body of in vitro evidence of

oligomeric Aβ interaction with endothelial [41–46] and smooth muscle cells [47, 48], and by post mortem studies showing coexisting cerebrovascular disease in most AD patients [6, 49–51].

Neurofibrillary tangles (NFT), a characteristic hallmark of AD together with senile plaques, have also been proposed to originate from chronic cerebral hypoperfusion [52], although manifesting in the later stage of AD progression [53]. However, there is limited quantitative evidence of a direct link between microvascular dysfunction and NFT formation, and microvascular abnormalities appear to correlate to Aβ deposition rather than neurofibrillary tangles [54, 55].

As early elements which could precede the clinical manifestation of AD by years or even decades [56–59], chronic cerebral hypoperfusion and BBB dysfunction emerge as a crucial topic of investigation, with prospective potential for therapeutic intervention.

1.1 Review focus

The endothelium-mediated processes implicated in the vascular component of AD pathogenesis interact in a complex network of cause-effect relationships. A comprehensive overview of this network is currently lacking, and could prospectively contribute to the identification of adequate therapeutic interventions.

This study reviews the current literature of in vitro and ex vivo studies on endothelium-mediated mechanisms underlying vascular dysfunction in AD pathogenesis (sections 2-4), with the aim of presenting a comprehensive overview of the network of causative relationships (section 5). Particular emphasis is given to mediators of detrimental vicious circles, such as hypoxia/ischemia, oxidative stress, inflammation and mitochondrial dysfunction. The potential role of pericytes and astrocytes in this complex scenario is also investigated.

2. BBB dysfunction in AD

In the cerebral circulation, the BBB is a highly specialized structure which maintains neuronal homeostasis by regulating the flux of electrolytes, metabolites, toxic molecules, xenobiotics, and circulating

immune cells between the bloodstream and the brain parenchyma [60, 61]. The BBB is formed by the capillary endothelium, the basement membrane, and the surrounding pericytes and astrocyte end-feet. The endothelial cells of the BBB adhere to one another through junctional structures, termed tight (TJ) and adherens junctions, which regulate paracellular permeability (the so-called 'gate function'), and maintain the polarity of enzymes and receptors on the luminal and abluminal domains of the endothelial membrane ('fence function') [34, 62]. Small lipid-soluble molecules, such as oxygen, carbon-dioxide, and typical therapeutic drugs, can diffuse freely across the BBB [63], whereas the exchange of larger molecules occurs by active transport (transcytosis) through the cell body or by paracellular transport [9, 64].

In AD, the BBB undergoes functional and structural changes which disrupt the gate function, impair energy supply to the brain, reduce the clearance of Aβ, and produce neurotoxic molecules. Due to its central role in cerebral homeostasis, the BBB becomes both a target and source of injury in the development of the disease.

2.1 Impaired glucose transport

Glucose – the main energy source for the brain – requires a carrier (transporter) to cross the BBB. In the human BBB, this is mainly achieved by glucose transporter 1 (GLUT-1).

Glucose uptake in the brain is determined by the concentration of glucose in the plasma, as well as the concentration of GLUT transporters in the BBB. Positron emission tomography (PET) studies have demonstrated reduced regional metabolic rate in the AD brain, especially in temporal and parietal cortical regions [65–68]. Impaired glucose transport across the BBB could contribute to this condition by acting as rate limiting factor [22], as suggested by autopsy studies showing reduced concentration of GLUT transporters in the microvasculature of the AD brain [23, 69, 70].

Among possible causes of impaired glucose transport, it has been proposed that mitochondrial dysfunction in the BBB endothelium could play an important role [22, 71]. In addition, the ultrastructural alterations observed in capillary walls of the AD brain [72–74] might – in principle – induce structural alterations of transport proteins [75], thus hindering the active transport of nutrients across the

BBB. However, it is also possible that this putative transport defect does not lower intra-cerebral glucose content to a sufficient extent to alter the metabolic rate [76, 77]. Furthermore, it remains unclear whether the hypothesised rate limiting effect of impaired transport could explain the early manifestation of hypometabolism observed in AD [78].

Taken together, the available evidence suggests, but does not prove, that impaired GLUT-dependent transport could contribute to the reduced glucose metabolism observed in AD, and this contribution could be aggravated by mitochondrial dysfunction and ultrastructural cellular damage.

2.2 Oxidative stress

At low concentrations, reactive oxygen species (ROS) participate in the regulation of cell functioning by activating intracellular signalling cascades, whereas at higher concentrations ROS may cause oxidative stress, a condition in which the production of ROS overcomes antioxidant defences [79], resulting in damage to lipids, proteins, and DNA [80].

Oxidative stress is a feature of aging [81, 82], and age-related diseases [83], including AD [26, 84, 85]. Due to its high rate of oxygen consumption, presence of redox-active metals and limited antioxidant enzymatic defences, the brain is particularly vulnerable to oxidative damage [80, 86], and increased levels of lipid peroxidation and nucleic acid oxidation are a consistent finding in the AD brain [72, 87].

Because Aβ plaques sequester redox-active metals [31], and Aβ deposits have been found in perivascular cells and perivascular spaces surrounding cortical microvessels [72], it has been suggested that the cerebral microvasculature, and in particular the BBB, could actively contribute to the oxidative injury observed in the AD brain. This view is supported by the highly reactive nature of the BBB endothelium, which is both a source of, and a target for, ROS and inflammatory proteins [18, 88]; and by the peculiar features of the ultrastructural damage in endothelial and perivascular cells, which is characterised by large lipid-laden vacuoles and damaged, swollen mitochondria [72]. Although a causative link between oxidative stress and microvascular damage has not been established in the AD brain in vivo, in vitro studies have shown that chronic oxidative stress increases BBB permeability,

promotes leukocyte adhesion, and alters endothelial signal transduction and redox-regulated transcription factors [89].

Taken together, these data suggest that the dysfunctional BBB could actively foster the neurodegenerative process through separate (and possibly synergistic) pathways, by increasing the levels of ROS in the brain, by promoting the extravasation of monocytes and toxic molecules into the perivascular space, and by further reducing regional perfusion in a vicious circle.

2.3 NO-mediated disruption of microvascular homeostasis

NO is an important regulatory molecule with a fundamental role in neurovascular homeostasis. In endothelial cells, NO regulates vascular tone, platelet aggregation, leukocyte adhesion, and endothelial junctional permeability [90–92]. NO is produced by three isoforms of NO synthase (NOS), endothelial (eNOS), neuronal, and inducible (iNOS). The latter is induced (also in endothelial cells) by transcription factors which are activated by cytokines during inflammation [93], and is capable of increasing overall NO production, far beyond the levels produced by eNOS [94].

A common feature of aging and cerebrovascular disease is the decrease of baseline endothelial NO synthesis [27]. Reduced endothelial NO production/bioavailability results in impaired vasodilation, reduced regional cerebral blood flow (CBF), and accumulation of oxidative stress, which are common features in AD [95]. There is evidence that pharmacological or genetic inactivation of eNOS in cultured brain microvascular endothelial cells increase the expression of AβPP and β-site AβPP cleaving enzyme 1 (BACE-1), as well as Aβ production [96]. Consistently with these findings, other studies [97, 98] have shown an inverse correlation between eNOS-positive capillaries and Aβ senile plaques in cortical samples of AD brains.

Chronic inhibition of constitutive NO production also increases endothelial permeability during inflammation [99]. Exposure of cultured human brain endothelial cells to cytokines (tumour necrosis factor (TNF)-α, interleukin (IL)-1β, interferon (IFN)-γ), decreased transendothelial electrical resistance (TEER) and increased TJs permeability [99], which could be reverted by NO donors.

Constitutive endothelial NO is reduced in chronic hypoperfusion, as evidenced by reduced eNOS immunostaining in rat hippocampal capillaries following chronic bilateral carotid occlusion [100], with concomitant evidence of mitochondrial damage in endothelial and perivascular cells. These abnormalities were associated with amyloid deposition surrounding the capillary wall, suggesting a possible interaction of the vascular damage with Aβ deposits. In addition, occluded rats (but not controls) showed worsened spatial memory following administration of eNOS inhibitors 8 weeks after occlusion. These findings suggest that vascular NO derived from eNOS may play an important role in regulating microvascular tone in the attempt to contrast the chronic reduction of CBF [100].

Collectively, these data indicate that endothelial NO dysregulation may contribute to BBB dysfunction and permeability, oxidative stress, chronic regional hypoperfusion, and increased Aβ production. The effects of reduced endothelial NO could also be increased during inflammation and chronic hypoperfusion in a detrimental vicious circle, which could accelerate the neurodegenerative process in AD.

2.4 Aβ-endothelium interaction

2.4.1 Aβ trafficking and impaired Aβ clearance

Aβ clearance from the brain is mediated by various mechanisms, such as glial phagocytosis, enzymatic degradation, transport to the cerebrospinal fluid with subsequent re-absorption into the venous circulation, and direct transport across the BBB [101].

As macromolecules, Aβ peptides cross the BBB by active transport. The receptor for advanced glycation end-products (RAGE) mediates Aβ transcytosis to the perivascular space (Aβ influx) [102, 103], whereas Aβ efflux is mediated by multiple receptors, and in particular the lipoprotein receptor-related protein 1 (LRP-1) [39, 104], and ATP binding cassette (ABC) subfamily B member 1, also termed P-glycoprotein (P-gp) [105–107]. Data from mouse models suggest that RAGE expression increases with age [108], whereas LRP-1 and P-gp decline [106], suggesting a possible path for in-

creased Aβ deposition, and decreased clearance, respectively. P-gp expression was also found to inversely correlate with the deposition of $A\beta_{1-40}$ and $A\beta_{1-42}$ in elderly non-demented humans [109], indicating a possible dose-dependent path for Aβ accumulation with increasing age.

Due to the potential implication of impaired Aβ transport across the BBB on the development of AD, this topic has attracted substantial research effort. While some biopsy studies have shown increased Aβ influx mediated by RAGE upregulation in the human AD brain [55, 110, 111], reports from animal studies are conflicting. On the other hand, evidence of impaired Aβ efflux appears to be more consistent, with an implication of P-gp and LRP-1 transporters [39].

Using PET imaging, P-gp function has been found to be reduced in AD patients [107], concomitant with increased Aβ deposition. This finding is consistent with a later in vitro study [112], in which $A\beta_{1-42}$ reduced P-gp expression in a murine BBB model. Interestingly, the Aβ-induced decrease of P-gp was attenuated when astrocytes were in close contact with endothelial cells, suggesting a protective role for astrocytes in preserving the expression of this transporter. As biopsy studies show morphological alterations of endothelial cells, the question arises whether these changes could "detach" astrocytes from the basement membrane of the BBB endothelium, creating a favourable condition for the inhibition of Aβ efflux transporters. This hypothesis appears to be supported by the observation that the above morphological alterations precede the formation of perivascular Aβ deposits [113]. Consistent with [112], another recent post mortem study [114] reported an inverse correlation between the burden of Aβ senile plaques and P-gp positive capillaries.

Biopsy studies also suggest that Aβ alters LRP-1 activity [55, 111, 115]. Owen and colleagues [115] proposed that Aβ could impair its own efflux from the brain by oxidising LRP-1, thereby progressively inhibiting its vascular clearance pathway. A different view was presented by Wilhelmus and colleagues [116] who found that Aβ increased LRP-1 expression, and proposed that LRP-1 uptake of Aβ could ultimately saturate, resulting in Aβ accumulation. There is also evidence that BBB-mediated clearance of Aβ could be modulated by ApoE, in an isoform-dependent manner. Data from mouse models suggest that ApoE (especially ApoE-ε4) could divert Aβ efflux to slower receptors than LRP – such as the very low density lipoprotein receptor – ultimately resulting in Aβ accumulation [117].

Taken together, the above data indicate a possible pathway of BBB-mediated Aβ accumulation, predominantly based on reduced efflux rather than increased influx across the barrier. However, the increase of influx transporters or decrease of efflux transporters only suggests a possible pathway of Aβ accumulation, it does not prove that the parenchymal accumulation of the peptide actually occurs because of this pathway [39], especially in vivo. Furthermore, it has recently been estimated that in the human brain, Aβ efflux across the BBB accounts for approximately 25% of the overall clearance of Aβ [118], whereas other pathways – including glial phagocytosis, proteolytic degradation, and transport to the cerebrospinal fluid with subsequent re-absorption into the venous blood [101, 119] – cumulatively account for the predominant proportion, suggesting that impaired BBB-mediated efflux might contribute to Aβ accumulation only marginally.

2.4.2 Endothelium-mediated mechanisms of Aβ production

It has been suggested that AβPP and Aβ observed in the perivascular space of the AD brain may be of endothelial origin [120, 121]. According to this hypothesis, altered expression of AβPP in endothelial cells could contribute to the accumulation of Aβ. Evidence from cultured endothelial cells suggests that thrombin could be implicated in this process by inducing AβPP secretion through both intracellular and cell surface pathways [122]. Prolonged endoplasmic reticulum (ER) stress could also induce intracellular AβPP accumulation and processing, leading to increased intracellular Aβ levels [123].

The possible effects of other stressors such as hypoxia, decreased glucose supply, and exposure to growth factors, on endothelial Aβ production have also been studied. Oxygen-glucose deprivation – an accepted model of ischemia [14, 124] – has recently been shown to increase $A\beta_{1\text{-}42}$ production in the rat brain capillary endothelium through the upregulation of BACE-1 mediated by hypoxia inducible factor 1α (HIF-1α) [120].

It has been suggested that endothelial cells in the BBB could also contribute to Aβ production through the activity of enzymes which would cleave AβPP retained by circulating platelets (whose number is increased in AD), leading to the formation of Aβ fragments [39]. This hypothesis, however, appears to be supported by limited experimental evidence.

Collectively, these data suggest a potential active role of the BBB endothelium in the production of Aβ through proteolytic processing of AβPP. However, the extent to which this phenomenon could contribute to the parenchymal accumulation of the peptide and to the neurodegenerative process remains to be established in vivo.

2.4.3 Aβ-induced BBB permeability and transmigration of mononuclear cells

Exposure of endothelial cells to Aβ induces morphological and biochemical alterations which affect BBB permeability. Deli and colleagues [125] reported $A\beta_{1-42}$-induced ultrastructural changes in cultured rat brain endothelial cells such as vacuolization, decreased number of caveolae and Golgi bodies, and shrunken mitochondria. Irregular interendothelial junctions with fewer points of contact were also observed, with decreased TEER and increased paracellular permeability to fluorescein and albumin. TJ integrity in mouse endothelial cells was also regulated by ApoE [126], and $A\beta_{1-42}$-RAGE interaction [127, 128]. Some line of evidence also suggests that Aβ-induced BBB permeability might be mediated by protein kinase C (PKC), a family of enzymes involved in transmembrane signal transduction. PKC participates in the regulation of ion and water transport in the brain by stimulating the (Na^+/K^+)-ATPase pump [129], and has been indicated as potential mediator of BBB permeability [44]. Alterations in this enzyme have been reported in aging and dementia [130]. In AD, the activity of PKC and its isoforms is reduced in the cortex and hippocampus [131–134], and also in cerebral microvessels [44, 135]. Endothelial cells exposed to $A\beta_{1–40}$, have shown PKC translocation from the plasma membrane to the intracellular domain (inactive state), suggesting that Aβ-endothelium interaction might contribute to PKC inactivation [136] and, ultimately, BBB permeability.

Aβ interaction with endothelial cells has also been shown to increase adherence and transmigration of monocytes across the BBB [43, 137, 138]. Transmigrated monocytes can then undergo differentiation into microglia [139], and become activated in inflammatory conditions.

In vitro studies have identified potential mechanisms by which Aβ could promote endothelial adhesion and transmigration of monocytes. Using a BBB model of human brain microvascular endothelial cells, Giri and colleagues [137] found $A\beta_{1–40}$-induced monocyte transmigration to be inhibited by

RAGE and platelet endothelial cell adhesion molecule (PECAM-1) antibodies, which suggests a mediating role of the Aβ influx transporter. A subsequent study from the same group [43] showed increased transmigration when the apical surface (luminal side) of the endothelium was exposed to $A\beta_{1-40}$, suggesting that cell polarity is another important factor.

Aβ aggregation state has also been shown to play a role. Isolated soluble Aβ aggregates activated adhesion and transmigration of monocytes, whereas un-aggregated monomers and mature fibrils did not [138]. A further study from the same group also demonstrated an implication of the nuclear factor-kB (NF-kB) in mediating Aβ-induced endothelial permeability to monocytes [140]. Aβ-induced BBB permeability to mononuclear cells has also been demonstrated in vivo. Farkas and colleagues [141] observed T-lymphocyte transmigration across the BBB after carotid infusion of $A\beta_{25-35}$ in rats.

In the perivascular space, the vasoactive agents and cytokines released by activated microglia can modify TJ assembly, further enhancing BBB permeability [34, 142] and the paracellular route for mononuclear cell extravasation [60]. This condition could trigger an "autotoxic" vicious circle in which the activation of microglia could cause neuronal damage, leading to further microglial activation [143, 144]. The latter effect appears to be sensitive to Aβ. Indeed, microglia exposed to $A\beta_{1-42}$ show increased expression of cytokines [145], suggesting that Aβ might exacerbate the effects of the vicious cycle.

In the AD brain, activated microglia have been shown to co-localize with perivascular deposits of Aβ [146], suggesting a possible implication in the neurodegenerative process. While early accumulation of microglia seem to play a neuroprotective role by promoting Aβ clearance [147], data from mouse models suggest that this neuroprotective effect might be unable to cope with persistent Aβ accumulation, especially in increasing age and progressing AD pathology [148], thus supporting the hypothesis that Aβ-induced monocyte transmigration could play a role in the neurodegenerative process.

Taken together, these data corroborate the hypothesis that activated microglia could contribute to exacerbating the inflammation-mediated neurodegenerative process of AD, especially under conditions of chronic inflammation and Aβ accumulation.

2.4.4 Vasoactive and apoptotic effects of Aβ

Aβ has vasoactive effects on endothelial cells in vitro, which might contribute to reducing cerebral perfusion in AD. Deane and colleagues [102] observed reduced cortical CBF following infusion of $A\beta_{1–40}$ in the mouse brain, which they showed to be Aβ-RAGE dependent and mediated by the upregulation of the vasoconstrictor endothelin-1 (ET-1). This vasoactive effect of Aβ was later confirmed by Palmer and colleagues [149] who reported $A\beta_{1-40}$- and $A\beta_{1-42}$-induced increases in ET-1 release in primary cultures of human brain endothelial cells.

Aβ has also been shown to cause endothelial cell death in vitro [42, 46, 150]. Exposure of cerebral endothelial cells to elevated (micromolar) doses of $A\beta_{1-40}$ caused mitochondrial dysfunction, nuclear and mitochondrial DNA damage, and cell death [46]. Consistent with these findings, a later study by Fonseca and colleagues showed the activation of ER stress-induced unfolded protein response (UPR) in rat brain endothelial cells incubated with $A\beta_{1–40}$. UPR caused Ca^{2+} leakage from the ER store into the cytoplasm, and the activation of both mitochondria-dependent and independent apoptotic pathways [42]. A further study from the same group showed $A\beta_{1–40}$-induced increases of nuclear HIF-1α, vascular endothelial growth factor (VEGF) and GLUT1, which correlated with oxidative stress markers [45]. Despite the above VEGF upregulation, several studies have shown the lack of angiogenesis [151–153], suggesting that in AD, Aβ-endothelial interaction could alter the ability of vessels to repair and regenerate after injury.

Aβ has also been shown to dysregulate endothelial NO production [154, 155], possibly through the alteration of cytosolic Ca^{2+} homeostasis caused by Inositol 1,4,5-triphosphate (IP_3) receptor leakage in the ER [154]. There is also evidence that the Aβ-induced reduction of NO bioavailability could be mediated by oxidative stress [156].

Collectively, the above in vitro evidence suggests that Aβ-endothelium interaction is able to disrupt intracellular Ca^{2+} homeostasis, with detrimental effects on NO synthesis, mitochondrial function, and ROS production, leading to accelerated cell senescence and death. These effects could in turn be aggravated by a chronic state of hypoperfusion, such as resulting from sustained ET-1-mediated microvascular vasoconstriction.

2.5 Endothelial response to chronic hypoperfusion-related hypoxia/ischemia

Chronic regional hypoperfusion in the brain has been shown to compromise memory processes [14]. Substantial evidence from in vitro and biopsy studies also supports the hypothesis that chronic cerebral hypoperfusion plays an important role in the development of AD [7, 72, 75, 157–159]. Hypoperfusion-induced hypoxia evokes vascular responses, in which endothelial cells in the cerebral microcirculation play a central role. In vitro and biopsy studies have shown that hypoxia modulates endothelial junctional permeability, ROS generation [160, 161] and stimulates pro-inflammatory gene expression [18, 162].

The transcription factor HIF-1α is a key regulatory mediator of cellular responses to hypoxia, acting as sensor of low oxygen tension [18]. HIF-1α is elevated in the cerebral microcirculation of AD patients and AD mouse models [163, 164], and several studies have reported elevated inflammatory proteins in brain endothelial cells exposed to hypoxia [18, 164], suggesting a link between hypoxia and cerebrovascular inflammation. This hypothesis is also supported by the work of Yamagata and colleagues [21] who reported a decrease in TEER of endothelial cells exposed to hypoxia, which was associated with IL-1β and NO.

Hypoxia also affects AβPP processing and Aβ production [165], which could further contribute to the development of AD pathology. Acute hypoxia increased the expression and activity of BACE-1, resulting in increased Aβ production [166]. Consistently with the above, exposure of cultured endothelial cells to ischemia stimulated AβPP expression and cleavage into Aβ, resulting in increased Aβ production [167].

Hypoxia [21, 25, 168–170] and ischemia [171] have been shown to induce BBB permeability by altering the expression of junctional proteins. Importantly, the combined effect of hypoxia and aglycemia appears to enhance the increased BBB permeability produced by hypoxia alone [172].

Hypoxia is also a potent stimulus for vascular activation and angiogenesis. Microvessels isolated from the brain of AD patients express a large number of angiogenic proteins, including VEGF [164], but without evidence of vascular growth [173]. The lack of vascular response could cause a chronic state

of activation of endothelial cells [174], resulting in the release of proinflammatory and potentially neurotoxic products.

Hypoxia might also contribute to the low levels of mesenchyme homeobox 2 (MEOX-2) – a transcription factor which regulates vascular cell differentiation and remodelling – which have been found in AD [175]. Downregulation of MEOX-2 in the AD brain endothelium have been shown to mediate an aberrant angiogenic response to VEGF resulting in vascular regression and reduced CBF [176]. Low levels of MEOX-2 also promote endothelial LRP degradation [176], thus favouring Aβ accumulation. Because of the anti-angiogenic effect of Aβ on brain endothelial cells [151, 153, 177], MEOX-2 might mediate a cooperative effect of hypoxia and Aβ to induce vascular regression, ultimately leading to reduced regional CBF.

Taken together, these data indicate multiple potential neurotoxic effects of chronic cerebral hypoperfusion, mediated by hypoxia/ischemia-induced activation of the BBB endothelium, resulting in Aβ production, expression of inflammatory proteins, BBB permeability, and vascular regression. The combined effects of hypoxia and aglycemia appear to cooperate in magnifying the endothelial response.

2.6 Microvascular endothelium as mediator of inflammatory processes

Due to their critical role as regulators of cerebral homeostasis, perturbations of the microvascular endothelium are closely linked to the pathophysiology of neuroinflammatory and neurodegenerative disease states, including AD [178–180].

Activated microglia and astrocytes are known endogenous sources of cytokines and chemokines in the AD brain [181]. However, increasing evidence shows that cerebral microvessels are also capable of expressing inflammatory mediators. Endothelial cells isolated from AD brains have revealed increased expression of iNOS [94], and intercellular adhesion molecule-1 (ICAM-1) [182]. Non-stimulated AD microvessels release higher levels of IL-1β, IL-6, TNF-α and transforming growth factor (TGF)-β compared to non-AD brains [181, 183]. In addition, cultured endothelial cells exposed to

oxidative stress have been shown to release thrombin [180], a neurotoxic protease which has been observed in cerebral microvessels [20] as well as senile plaques and NFT [184]. Thrombin, in turn, mediates the endothelial response to hypoxia by upregulating HIF-1α, inflammatory proteins (monocyte chemoattractant protein (MCP)-1, IL-6), matrix metalloproteinase-2, and ROS [18]. Inflammation and hypoxia have also been shown to cooperate in mediating BBB permeability [21].

Collectively, these data suggest that the cerebral microvasculature could actively contribute to the inflammatory process observed in AD, contributing to the release of neurotoxic agents and increasing endothelial permeability. However, whether inflammation contributes to AD pathogenesis or it is a neurovascular response to the developing neurodegenerative process, remains unclear [6].

2.7 Mitochondrial dysfunction and damage in BBB endothelial cells

The ATP-dependent transport of macromolecules across the BBB requires efficient mitochondrial function for ATP production, which is reflected by the high number of mitochondria in endothelial cells of brain capillaries [9, 75]. However, data from autopsy studies of AD brains show reduced mitochondrial density in the endothelium of cerebral capillaries [73, 74], as well as mitochondrial damage – evidenced by broken cristae, membrane disruptions, swelling, and mitochondrial DNA deletions – which co-localised with amyloid depositions and atherosclerotic lesions [72].

Structural as well as functional mitochondrial alterations may result from different and possibly interrelated causes, such as aging, oxidative stress, and Aβ toxicity.

2.7.1 The effect of aging

Aging – the main risk factor for sporadic AD [50, 185] – is a recognized cause of progressive mitochondrial damage [80, 186, 187]. Aging cells are affected by increasing oxidative stress and perturbed energy homeostasis [86, 187]. Mitochondria play a central role in this process as source of cellular energy (through the production of ATP), as major contributors to ROS production, and regulators of apoptosis [187]. Alterations of mitochondrial function are generally associated with an impaired elec-

tron-transport chain [187–193]. Mutations of mitochondrial DNA (mtDNA) also accumulate with advancing age, possibly because of the proximity of mtDNA to the respiratory chain (the major site of oxidative stress), and a deficient mtDNA repair mechanism [187].

2.7.2 Effects of oxidative stress

Chronic hypoperfusion causes glucose and oxygen deprivation. These conditions in turn lead to increased ROS production through the disruption of the mitochondrial respiratory chain and subsequent depletion of ATP, elevation of matrix Ca^{2+} concentration, and release of cytochrome C [194].
Mitochondria, however, are not only sources of ROS and oxidative stress, but also a major target of oxidative damage. Lipids and proteins in the inner mitochondrial membrane are highly susceptible to ROS, and lipid peroxidation has been observed in oxidative injury [187, 195]. Damage to the inner membrane proteins and/or lipids can result in membrane depolarization and impaired mitochondrial function [82]. In the AD brain, oxidative injury has been demonstrated in mitochondria of neuronal [82, 187, 196], and also in vascular (endothelial) and perivascular cells [72, 89, 197]. Cellular energy failure is demonstrated in the AD brain by post mortem studies showing deterioration of mitochondrial ultrastructure, formation of non-mature mitochondria ("hypoxic mitochondria" [72, 89]) and excessive mtDNA deletions [72, 195].

2.7.3 The effect of Aβ

In addition to age and oxidative stress, structural and functional mitochondrial alterations may result from the interaction with soluble Aβ peptides [46, 198, 199], preceding the formation of Aβ plaques [200]. Aβ interaction with mitochondria has been shown to inhibit or reduce the activity of the respiratory chain complex III [201], complex IV [199–201], cause mtDNA damage [46], and activate apoptotic pathways [202]. Atamna and Frey [189] proposed a paradigm of Aβ-mediated mitochondrial dysfunction according to which Aβ would bind to heme and cause the loss of complex IV and subsequent ATP deficit. According to this paradigm, due to its peroxidase activity the Aβ-heme complex

would cause oxidative damage to endothelial cells, contributing to endothelial dysfunction and ultimately BBB disruption [189].

2.7.4 Consequences of deficient ATP production

Mitochondrial dysfunction results in deficient ATP production, which impairs ATP-dependent transport mechanisms in the plasma and ER membranes, such as the sodium (Na^+/K^+-ATPase) and calcium (Ca^{2+}-ATPase) pumps [9, 203], and the ABC transporters [9]. Impaired Na^+ and K^+ homeostasis in the perivascular space influences cell membrane depolarization in neurons, affecting neuronal and synaptic functions [9].

Impaired sodium and calcium pump function results in the dysregulation of intracellular calcium concentration and signalling. This in turn affects endothelial NO synthase (eNOS) activity [204] and BBB permeability [203]. The deficient ATP production of dysfunctional mitochondria also leads to the dysregulation of ABC transporters, affecting brain supply of glucose and other nutrients, as well as Aβ clearance from the perivascular space [9]. ATP deficiency also results in the activation of apoptosis [187].

ATP deficiency, however, does not only affect transport (and ion flux) across the cell and ER membranes. The ATP-sensitive potassium channels (mitoK_{ATP}) expressed in the mitochondrial membrane, are also affected in dysfunctional mitochondria. In the brain, mitoK_{ATP} are found at much greater concentrations than in cardiac myocytes, suggesting an important role of these channels in cerebral homeostasis [194]. In normal conditions, the opening of mitoK_{ATP} causes a net influx of K^+ into the mitochondrial matrix, resulting in membrane depolarisation (which reduces the driving force for Ca^{2+}-uptake, thus opposing Ca^{2+} accumulation [205] and excessive ROS production [206, 207], with acute neuroprotective effect [207, 208]) and accelerated electron transfer in the respiratory chain, resulting in increased ATP production [194]. Preserving this mechanism through the administration of diazoxide – a mitoK_{ATP} channel opener – in conditions of chronic hypoperfusion or ischemia/reperfusion injury has been shown to protect neural function [194] and the BBB barrier function [209] in vivo.

Taken together, ATP deficit causes the alteration of many key elements of cellular homeostasis, resulting in accelerated cell senescence and death. ATP deficit also impairs Aβ clearance and nutrient supply to the brain, thereby contributing to neuronal damage and AD progression. However, the majority of available quantitative data on mitochondrial dysfunction/damage relate to neuronal cells, and only relatively few studies have investigated the phenomenon in vascular cells in relation to AD.

2.8 Endothelial interaction with pericytes

Adjacent to the basement membrane of BBB endothelial cells are pericytes, multifunctional cells which contribute to structural stability, regulation of capillary blood flow, and clearance of toxic by-products [210, 211]. Cross-talk signalling pathways between endothelial cells and pericytes have been identified [38, 210, 212], which regulate proliferation, migration, and recruitment of pericytes. In light of these findings, it has been hypothesised [63] that the location of pericytes along the microvascular tree could be determined functionally by the cross-talk with the adjacent endothelial cells. This hypothesis is consistent with recent in vivo observations of adaptive plasticity of the cerebral microvasculature in the rat brain [213].

Pericytes have typical properties of the contractile apparatus and their cell membrane expresses receptors for multiple vasoactive mediators [38, 214–217]. The resting membrane potential of pericytes also appears to be regulated by similar mechanisms to those observed in vascular smooth muscle cells, with Ca^{2+}-activated potassium channels, L-type voltage-dependent Ca^{2+} channels, agonist-activated Ca^{2+} channels, and capacitative calcium entry [216]. Intracellular elevation of Ca^{2+} – a phenomenon which in muscle cells precedes contraction – has also been observed in stimulated pericytes [215–217].

At present, evidence of actual contraction is limited, and predominantly based on in vitro data. However, a recent study by Hall and colleagues [37] demonstrated that capillaries of anaesthetised mouse somatosensory cortex dilate in response to neuronal activity, as a result of pericyte relaxation. Dilation of capillaries was found to precede that of the feeding arterioles, suggesting an active role of pericytes in the hyperaemic response. The authors hypothesised that the relaxation of pericytes could be

induced by prostaglandin E2 or a related compound, also involving NO production to contrast the synthesis of the vasoconstrictor 20-hydroxyeicosatetraenoic acid. Whether arterioles receive a signal to dilate from pericytes or from vasoactive messengers, remains to be elucidated. The study also showed that when exposed to ischemia, pericytes first caused vasoconstriction of capillaries then died in rigor. Although this would be expected to cause a long-lasting increase of the local resistance to flow, the latter was not measured in the above study.

In summary, a potential role of pericytes in the regulation of regional CBF is starting to emerge, which could shed new light on microvascular dysregulation in pathological conditions in which chronic hypoperfusion and microvascular structural/functional damage are present. Although a link with AD has not been established [38], pericyte loss in mice overexpressing AβPP accelerates amyloid angiopathy, and pericyte deficiency leads to the development of tau pathology and early neuronal loss [210], suggesting a potential role of these scaffolding cells in AD development.

2.9 Endothelial interaction with astrocytes

Endothelial cells of the BBB are surrounded by perivascular astrocytic end-feet. In vitro evidence suggests that astrocytes may influence the BBB phenotype in the endothelium [218, 219]. Data from cultured cells demonstrate that astrocytes can modulate TJs (physical barrier) [34, 75, 220, 221], the expression and localization of transendothelial transporters, including P-gp and GLUT-1 among others [34, 222]. Astrocytes also upregulate the expression of transferrin receptor and transcytotic mechanisms for low-density lipoproteins [223], and secrete TGF-β, glial-derived neurotrophic factor, basic fibroblast growth factor, and angiopoetin 1, which have been shown to induce aspects of the BBB phenotype in endothelial cells [34]. Furthermore, among agents modifying endothelial function and BBB permeability, several can be released by astrocytes, such as ET-1, glutamate, cytokines (IL-1β, IL-6, TNF-α), and macrophage inflammatory proteins [222].

While the above data support the hypothesis of an inductive influence of astrocytes on the endothelial phenotype in the BBB, reciprocal influences may also exist [34, 224]. The perivascular end-feet of astrocytes show several specialized features, including the water channel aquaporin 4 (AQP4) and the

inward rectifier (Kir4.1) K^+ channel, which are involved in ion and volume regulation [34]. AQP4 co-localizes with Kir4.1 and is segregated by agrin (a large proteoglycan in the basal lamina of the BBB endothelium) to the perivascular astrocytic end-feet [34], suggesting that the endothelium may contribute to specialising the astrocytic phenotype [34].

Collectively, the above evidence suggests a complex interaction between astrocytes and BBB endothelial cells, with potential implications in pathological conditions where the homeostatic function of the BBB is impaired. Indeed, astrocytes contribute to increase BBB permeability during inflammation by releasing inflammatory cytokines [34, 35], and in vitro astrocytes amplify the endothelial response to ischemia by increasing junctional permeability, and adhesion molecule expression [225]. Although these data suggest a possible implication of astrocyte-endothelium interaction in AD pathogenesis, this link has not been established.

3. Microvascular innervation in the AD brain

3.1 Intrinsic microvascular innervation and CBF regulation

Cholinergic neurons of the basal forebrain and medial septum provide the major source of cholinergic intrinsic innervation to the cortex and hippocampus [226, 227]. The cholinergic cortical vasodilatation induced by stimulation of the basal forebrain is mediated by NO production, which is thought to reflect the activation of nitrergic interneurons and perivascular acetylcholine release [228]. Dysfunction of basal forebrain cholinergic neurons is a characteristic feature of AD [75, 228], which results in denervation of cortical microvessels [229, 230], reduced expression of eNOS [231], and reduced amount of nitrergic interneurons [230]. This condition likely compromises the ability of cortical perfusion to adapt to the increased metabolic demand caused by neuronal activation [229, 230], resulting in depressed CBF regulation, and ultimately, cerebral hypoperfusion. Experimental evidence supports the hypothesis that the basal forebrain can participate in neocortical CBF regulation, as it has been shown that unilateral lesions of the basal forebrain are followed by reduced ipsilateral CBF [75].

Taken together, the above data suggest that the loss of cortical cholinergic innervation might play a role in the regional CBF reduction observed in AD. However, it is unclear whether this phenomenon would contribute to the neurodegenerative process from an early stage (promoting a vicious circle), or only become manifest at advanced stages.

3.2 Microvascular adrenergic innervation and BBB permeability

Adrenergic receptors exist in brain microvessels, with a predominance of β receptors [232–237]. Increased levels of β_2 and α_2 adrenoceptors have been reported in AD [237], suggesting possible alterations of the extrinsic regulation of the microvascular tone, which might contribute to impairing regional CBF distribution. Because microvascular noradrenergic innervation in cerebral microvessels also participates in the regulation of the (Na^+/K^+)-pump [238] (an important feature of the BBB function which regulates water and electrolyte homeostasis) it could be hypothesised that alteration of noradrenergic innervation could also alter fluid exchange at the BBB, thus creating another potential pathway of impaired CBF distribution. However, quantitative evidence is needed to confront this hypothesis.

4. Microvascular ultrastructural alterations in the AD brain

Substantial evidence from post mortem biopsy studies shows ultrastructural alterations in cerebral microvessels of the cortex of the AD brain [74, 75, 239, 240], which appear to co-localise with regions of Aβ deposits [72]. Kalaria and Hedera [240] found capillaries with collapsed or degenerated endothelium in AD, which were almost absent in brain regions free of Aβ deposits and in control subjects. Another study from the same group [239] reported an increased content of collagen IV – the main constituent of the basement membrane – in microvessels of AD brains compared to age-matched controls. This finding is consistent with the work of Farkas and colleagues [241] who observed a thickened basement membrane and collagen accumulation in cortical microvessels of AD brains. Similar findings were described by Claudio [73], who additionally reported an inverse correlation between

endothelial pinocytotic vesicle concentration and mitochondrial concentration. Evidence of increased size/concentration of endothelial vesicles in AD has also been reported in other studies [72, 75]. Consistent with Claudio [73], Stewart and colleagues observed a reduced density of mitochondria in the cerebral capillary endothelium of AD patients [74]. The study also reported an increased number of capillaries containing pericytes, which the authors interpreted as a protective scaffold to support the weak endothelium which showed junctional leakiness. An interesting view proposed by Broadwell and Salcman [242] is that pericytes could also limit the damage of a leaky BBB by acting as macrophages, degrading extravasated serum proteins in their lysosomes.

Aliev and colleagues observed heterogeneous lesions in endothelial cells of cerebral microvessels of AD brains (and transgenic mice overexpressing AβPP), which were absent in age-matched controls [72, 89, 113, 197]. These vascular abnormalities included clusters of mitochondria-derived lysosomes, large-sized lipid vacuoles, and necrotic structures. Interestingly, in early AD samples without ultrastructural damage, the luminal side of endothelial cells protruded into the vessel lumen, suggesting that the effects of hypoperfusion might precede ultrastructural damage [89]. Furthermore, the ultrastructural abnormalities of the vascular wall cells co-localised with Aβ deposits around the microvessels [72, 89, 197]. Immunocytochemical analyses also revealed the presence of atherosclerotic lesions and mtDNA deletions in the damaged vascular wall, which were accompanied by increased AβPP and oxidative stress markers [89]. These vascular abnormalities were associated with the selective damage to cortical neurons [89, 197], suggesting that chronic hypoperfusion might be a primary cause of the accumulation of oxidative injury products [95], which would initiate the process of neurodegeneration. This hypothesis appears to be supported by the notion that oxidative stress is an early event in AD pathogenesis [72, 87, 243].

5. Network of causative relationships

Cerebral hypoperfusion and BBB dysfunction are key elements in the vascular pathway to AD. In vitro findings supports the hypothesis that factors resulting from cerebral hypoperfusion and BBB dysfunction are also potential causing factors, suggesting the existence of detrimental vicious circles.

The complex interconnections of endothelium-mediated mechanisms reviewed in previous sections are synthesised here in the form of a network of causative relationships linking the above mechanisms (Figure 1).

5.1. Chronic cerebral hypoperfusion

Aging (the main risk factor for sporadic AD [50, 185]), vascular conditions [6, 8–11], and possibly cardiac conditions [7, 12, 13] cause chronic cerebral hypoperfusion. This in turn reduces oxygen and energy supply to the brain tissue [14], ultimately leading to neuronal damage.

Hypoperfusion also damages the BBB and is aggravated by vasogenic oedema such as caused by pericyte detachment [9] or vascular cell death. Basal forebrain cholinergic deficit (a characteristic feature of AD [75, 228]) results in denervation of cortical microvessels [229, 230], further impairing CBF distribution. Hypoperfusion is also aggravated by the ischemia-induced death of pericytes [37], which constrict in rigor before dying, increasing resistance to microvascular flow.

Indirect effects of chronic hypoperfusion such as oxidative stress, inflammation and mitochondrial dysfunction, cause BBB permeability and structural damage to the microvascular wall [72]. These effects could mediate vicious circles, which could aggravate regional CBF reduction (and reduction of oxygen/glucose supply to the brain), ultimately accelerating the neurodegenerative process.

5.2 Hypoxia/ischemia

Hypoxia induces BBB permeability [21, 25, 168–170], ROS generation [160, 161] and stimulates pro-inflammatory gene expression [18, 162]. Hypoxia and inflammation may cooperate in inducing BBB permeability [21]. Likewise, aglycemia aggravates BBB permeability induced by hypoxia [172].

Hypoxia/ischemia affect AβPP processing and Aβ production [165–167]. Hypoxia also stimulates endothelial angiogenic proteins, including VEGF [164]. However, the lack of vascular growth in response to VEGF [173] might cause a chronic state of endothelial activation, resulting in the release of proinflammatory and potentially neurotoxic products. Hypoxia is also associated with low levels of MEOX-2 in AD, resulting in degradation of endothelial LRP-1, regression of capillary networks, and reduced cerebral microcirculation [175, 176]. Reduced LRP-1 promotes perivascular accumulation of Aβ. Because of the anti-angiogenic effect of Aβ [151, 153, 177], there may be a cooperative effect of hypoxia and Aβ to induce vascular regression and subsequent reduction of regional CBF, in a vicious circle.

Ischemia causes pericyte death and detachment [37]. The detachment of pericytes causes leakage of serum proteins and focal microhaemorrhages [9], resulting in vasogenic oedema [9], further contributing to hypoperfusion and hypoxia of the surrounding parenchyma, thus aggravating neuronal injury. Pericyte death in rigor causes a restriction of the capillary lumen [37], with increased resistance to flow and consequent reduction of regional CBF.

5.3 Oxidative stress

Excessive ROS and RNS production in oxidative stress causes scavenging of NO (e.g. by sequestration into peroxynitrite), with subsequent reduction of NO bioavailability [244]. Oxidative stress also damages mitochondria [82, 86]. Because mitochondria are a major source of ROS and dysfunctional mitochondria can increase ROS production [196], this might lead to a vicious circle which could aggravate BBB damage.

Oxidative stress also decreases the expression of LRP-1 and upregulates RAGE, potentially leading to increased perivascular Aβ deposition [15]. Furthermore, oxidative stress increases BBB permeability, promotes leukocyte adhesion [89], and endothelial release of the neurotoxic protease thrombin [180].

5.4 Inflammation

Endothelial cells isolated from AD brains release inflammatory proteins [94, 181–183]. Inflammation enhances the endothelial response to hypoxia increasing BBB permeability. Astrocytes also contribute to BBB permeability during inflammation by releasing inflammatory cytokines [34, 35].

5.5 Mitochondrial dysfunction

Glucose and oxygen deprivation caused by chronic hypoperfusion [194], oxidative stress [82, 86], and Aβ [46, 189, 198–202] contribute to the dysregulation of mitochondrial function. This in turn depletes ATP stores, impairs ATP-dependent transport (of ions and Aβ), and increases intracellular Ca^{2+} concentration, disrupting vital signalling pathways, and leading to apoptosis [9, 203]. Dysfunctional mitochondria also increase ROS production and oxidative damage in a vicious circle.

5.6 Aβ-endothelium interaction

Aβ efflux transporters P-gp and LRP-1 decrease in AD [107, 115], suggesting a possible pathway to perivascular Aβ accumulation. However, this contribution is likely marginal [118].

Aβ dysregulates endothelial NO production [154, 155], possibly through the alteration of cytosolic Ca^{2+} homeostasis [154]. Aβ-induced reduction of NO bioavailability might also be mediated by oxidative stress [156]. Inactivation of eNOS increases the expression of AβPP and BACE-1, as well as Aβ production [96], potentially sustaining a vicious circle. Reduced endothelial NO production/bioavailability in turn impairs regional CBF [100].

Aβ-endothelium interaction induces BBB permeability [125–128], possibly mediated by PKC inactivation [136]. Aβ also increases adherence and transmigration of monocytes across the BBB [43, 137, 138], mediated by the transcription factor NF-kB [140]. Transmigrated monocytes undergo differentiation into microglia [139], and become activated in inflammatory conditions. The vasoactive agents and cytokines released by activated microglia can in turn induce BBB permeability [34, 142], creating a paracellular route for mononuclear cell extravasation [60], in a vicious circle.

Furthermore, Aβ induces vasoconstrictor ET-1 release [102, 149] – thus reducing regional CBF [102] – and activates the endothelial inflammatory response [245, 246]. Finally, at high concentrations Aβ causes endothelial apoptosis [42, 46, 150] by disrupting intracellular Ca^{2+} homeostasis.

5.7 BBB permeability

BBB permeability is influenced by hypoxia/ischemia [21, 25, 168–170, 172], inflammation [19], oxidative stress [15], and Aβ [125–128]. Astrocytes also contribute to BBB permeability during inflammation by releasing inflammatory cytokines [34, 35], and amplify the endothelial response to ischemia by increasing junctional permeability [225].

Alterations of the adrenergic microvascular innervation might alter the (Na^+/K^+)-pump function of the BBB, possibly resulting in fluid balance impairment [238] and dysregulation of regional CBF.

6. Conclusions

Based on the current literature on endothelium-mediated mechanisms underlying vascular dysfunction in AD pathogenesis, this study has presented a comprehensive overview of the complex network of the interacting mechanisms, with particular emphasis on causative relationships, and mediators of detrimental vicious circles, such as hypoxia/ischemia, oxidative stress, inflammation and mitochondrial dysfunction. The possible implication of other components of the BBB, such as pericytes and astrocytes, in this complex scenario has also been investigated.

As new mechanisms are discovered in this rapidly evolving field of research, the proposed network can be expanded, adding new elements (mechanisms) and/or interactions. Prospectively, this network could be exploited in the identification of adequate therapeutic interventions to treat, delay or prevent AD.

Acknowledgement

This study was partially funded by the European Union's Seventh Framework Programme (FP7/2007 – 2013) as part of the project VPH-DARE@IT (grant agreement no. 601055); and by the Engineering and Physical Sciences Research Council (EPSRC) (OCEAN Project, Grant No. EP/M006328/1). Dr Farkas is supported by the Bolyai János Research Scholarship of the Hungarian Academy of Sciences (BO/00327/14/5). There are no conflicts of interest associated with this work.

Figure Captions

Figure 1. **Causative relationships of endothelium-mediated mechanisms of vascular dysfunction in AD pathogenesis**. Green boxes indicate systemic factors causing regional cerebral hypoperfusion, orange boxes indicate key mediators of vicious circles leading to hypoperfusion aggravation. Arrows indicate causal relationship in the direction of the arrow. Arrows pointing up inside a box indicate upregulation/increased quantity; arrows pointing down indicate the opposite. See text for further detail. ABC: ATP binding cassette (transporter); Aβ: amyloid-β; AβPP: Aβ precursor protein; BACE-1: β-site AβPP Cleaving Enzyme 1; BBB: blood-brain barrier; BF: basal forebrain; ER: endoplasmic reticulum; LRP-1: low-density lipoprotein receptor-related protein 1; MEOX-2: mesenchyme homeobox gene-2; NO: nitric oxide; PKC: protein kinase C; P-gp: P glycoprotein; RAGE: receptor for advanced glycation end-products; RNS: reactive nitrogen species; ROS: reactive oxygen species; TEER: Transendothelial resistance; VEGF: vascular endothelial growth factor.

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