Molecular Psychiatry (2014) 19, 159–167

www.nature.com/mp

REVIEW

# The neglected co-star in the dementia drama: the putative roles of astrocytes in the pathogeneses of major neurocognitive disorders

WK Jo[1], ACK Law[1,2,3] and SK Chung[3,4]

Alzheimer's disease (AD) and vascular dementia are the major causes of cognitive disorders worldwide. They are characterized by cognitive impairments along with neuropsychiatric symptoms, and that their pathogeneses show overlapping multifactorial mechanisms. Although AD has long been considered the most common cause of dementia, individuals afflicted with AD commonly exhibit cerebral vascular abnormalities. The concept of mixed dementia has emerged to more clearly identify patients with neurodegenerative phenomena exhibiting both AD and cerebral vascular pathologies—vascular damage along with β-amyloid (Aβ)-associated neurotoxicity and τ-hyperphosphorylation. Cognitive impairment has long been commonly explained through a 'neuro-centric' perspective, but emerging evidence has shed light over the important roles that neurovascular unit dysfunction could have in neuronal death. Moreover, accumulating data have been demonstrating astrocytes being the essential cell type in maintaining proper central nervous system functioning. In relation to dementia, the roles of astrocytes in Aβ deposition and clearance are unclear. This article emphasizes the multiple events triggered by ischemia and the cytotoxicity exerted by Aβ either alone or in association with endothelin-1 and receptor for advanced glycation end products, thereby leading to neurodegeneration in an 'astroglio-centric' perspective.

*Molecular Psychiatry* (2014) **19,** 159–167; doi:10.1038/mp.2013.171; published online 7 January 2014

**Keywords:** Alzheimer's disease; astrocytes; ET-1; ischemia; RAGE; vascular dementia

## INTRODUCTION

### Alzheimer's disease and vascular dementia

Alzheimer's disease (AD) has constituted a major public health challenge for the elderly population worldwide. The prevalence of AD increases significantly with age and comprises 50–70% of all dementia cases.[1,2] AD is histopathologically characterized by the formation of β-amyloid (Aβ) and neurofibrillary tangles, leading to neuropathological events and subsequent synaptic dysfunction, glial activation and eventual neuronal demise.[3–6]

Vascular dementia (VaD) is another common cause of dementia, accounting for 15–25% of all dementia cases.[1] It is characterized by cerebrovascular dysregulations such as a decrease in the cerebral blood flow (CBF), breakdown of the blood brain barrier (BBB), upregulation of inflammatory processes and the production of reactive oxidative species (ROS), leading to multiple lacunar infarcts, leukoencephalopathy, and hemorrhagic and ischemic lesions, thereby resulting in neurodegeneration.[7] VaD is characterized into three main subtypes: cortical VaD—also referred to as multi-infarct or post-stroke dementia, subcortical VaD or small-vessel dementia and strategic-infarct dementia. Other subtypes include hypoperfusion dementia, hemorrhagic dementia and hereditary VaD. Cortical VaD is characterized by multiple cortico-subcortical infarcts related to large vessel disease and major hemodynamic events. Subcortical VaD is related to small-vessel disease and is characterized by small infarcts causing lacune development and ischemic white matter lesions with incomplete ischemic injuries. Strategic-infarct dementia is characterized by ischemic lesions that occur in specific regions critical for higher cognitive functions.[8–10]

Cerebrovascular abnormalities are often found to co-exist in the AD brains, thereby blurring the distinction between AD and VaD. Mixed dementia (MixD)—individuals having both AD and VaD pathologies—has therefore been conceptualized.[11–15] The clinical criteria being used to identify AD, VaD and MixD, including the Consortium to Establish a Registry for AD, Alzheimer Disease Diagnostic & Treatment Center, National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neuroscience, among others, show inconsistency, thereby yielding highly variable prevalence rates of 2 to 60% for MixD.[16–19]

Some of the major vascular pathological features found in AD with cerebrovascular abnormalities include cerebral amyloid angiopathy, intracerebral Aβ-deficient clearance through the BBB and ischemic stroke lesions, suggesting the significant involvement of the cerebral vasculature in AD pathophysiology.[20,21] The neurovascular unit (NVU) can be considered as the smallest functional component of the neurovasculature, comprising of endothelial cells, pericytes, vascular smooth muscle cells and with the astrocytes end-feet being the direct links between neurons and the cerebral vasculature.[22] The NVU functions to control CBF, BBB exchange, transmigration of leukocytes in brain

[1]Neural Dysfunction Research Laboratory, Department of Psychiatry, LKS Faculty of Medicine, The University of Hong Kong, Pok Fu Lam, Hong Kong; [2]Research Centre of Heart, Brain, Hormone and Healthy Aging, LKS Faculty of Medicine, The University of Hong Kong, Pok Fu Lam, Hong Kong; [3]State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Pok Fu Lam, Hong Kong and [4]Department of Anatomy, LKS Faculty of Medicine, The University of Hong Kong, Pok Fu Lam, Hong Kong. Correspondence: Dr ACK Law, Neural Dysfunction Research Laboratory, Department of Psychiatry, LKS Faculty of Medicine, The University of Hong Kong, Room 219, Block J, Queen Mary Hospital, 102 Pok Fu Lam Road, Pok Fu Lam, Hong Kong.
E-mail: acklaw@hku.hk
Received 1 August 2013; revised 26 September 2013; accepted 29 October 2013; published online 7 January 2014

infection and the trophic interaction between the endothelial cells, neurons and glia.[23]

Research has shown that intracerebral Aβ is transported outside by the low-density lipoprotein receptor-related protein 1 and transported into the brain by the receptor for advanced glycation end products (RAGE), with both receptors being localized on the BBB.[23] Interestingly, disruption of NVU homeostasis secondary to chronic ischemia could lead to a decrease in CBF, disrupting the BBB integrity, thereby leading to the formation of Aβ plaques, trophic support impairment, also triggering oxidative stress and inflammation.[24–28] Taken together, NVU dysfunction is likely to be an important early pathological event that leads to neurodegeneration as observed in AD and MixD.

## ASTROCYTE: MULTIPLE FACETS

Comprehensive literature has depicted the pivotal roles of astrocytes in the neurological disorders over the previous decades. They have been implicated in a number of neurodegenerative diseases, including AD, amyotrophic lateral sclerosis, Wernicke encephalopathy, frontotemporal dementia, frontotemporal lobar degeneration, thalamic dementia and Huntington's disease.[29] They appear to have significant, yet controversial, roles in the central nervous system (CNS) pathophysiology.

### Anatomical features

Astrocytes are star-shaped cells that exist in well-distributed, non-overlapping fashion in the CNS, each confined in its own discreet territory.[30,31] Interdigitation primarily occurs at the end tips of their diversified processes, forming gap junctions with adjacent astrocytes, establishing close contact with neurons and blood vessels, and enveloping thousands of synapses.[32] The two main classical subtypes are the protoplasmic and fibrous astrocytes.[33] Protoplasmic astrocytes are found in the gray matter with many fine processes enveloping synapses, whereas fibrous astrocytes are within the white matter, extending longer but with fewer processes to the nodes of Ranvier.[32] Further morphological studies have provided more diversified subdivisions, including Bergmann glia, Müller glia, tanycytes, radial, velate and perivascular astrocytes.[34]

### Physiological functions of astrocytes

Initially regarded as supportive elements in the brain, astrocytes are now known to have multiple neurophysiological roles. Astrocytes participate in the development of the neuronal circuitry by being actively involved in synaptic formation, maturation and pruning. Astrocytes also secrete several synaptogenic molecules, including thrombospondin, brain-derived neurotrophic factor (BDNF) and glypican.[35] They are responsible for the uptake and release of numerous transmitters important for information signaling, such as glutamate, γ-aminobutyric acid, purines, D-serine, acetylcholine, prostaglandins and eicosanoids.[36] Moreover, astrocytes are able to establish bidirectional communication with neurons, modulating synaptic information transmission, presynaptic and postsynaptic events, and are commonly known as the 'tripartite synapse'.[37]

Modulation of $Ca^{2+}$ signaling is another central task for astrocytes. Regulation of CBF by astrocytes is attributed by either the release of cyclooxygenase 1 metabolites through activation of phospholipase A2, or an alternative pathway involving the release of $K^+$ through the $Ca^{2+}$-activated $K^+$ channels into the extracellular space between astrocytic end feet and the arteriole in order to induce vasodilation.[38,39] Elevation of intracellular $Ca^{2+}$ concentration is likewise needed for the release of excitatory and inhibitory 'gliotransmitters' such as glutamate and adenosine, respectively.[40] In addition, excess of glutamate in the extracellular space is also neurotoxic to the brain, and astrocytes constitute the primary CNS cell type for glutamate clearance from the extracellular space through its uptake by the excitatory amino acid transporters 1 and 2, and convert it to glutamine by the enzyme glutamine synthetase.[41]

Astrocytes also accomplish many other responsibilities in the CNS, including anatomically ensheathing the BBB, influencing its permeability by inducing junction formation and the upregulation of several transporter enzymes on the BBB such as glucose transporter 1 and P-glycoprotein.[42] Water and $K^+$ homeostasis in the brain is also achieved by the coupling of both aquaporin 4 and rectifying $K^+$ channels localized on astrocytic end-feet.[43]

### Astrocytic reactivity

Astroglial responses in CNS pathologies are often mediated by astrogliosis, a process that leads to astrocyte morphological changes characterized by an augmented expression of intermediate filaments, proliferation and astrocytic hypertrophy surrounding the affected area.[44] The main intermediate filaments of the astrocytic cytoskeleton that are increased in astrogliosis are glial fibrillary acidic protein (GFAP), vimentin and nestin.[45] Hypertrophy is resulted from the somata and processes thickening, and it appears that interdigitation is minimal and domain overlap does not occur.[46] However, this is only partially true depending on the experimental model being studied; loss of domain architectural structures is reported in the brains of epileptic mice, whereas this is not the case for AD animal models.[47] Interestingly, recent research has discovered that distance from the lesion has a crucial role in determining the morphological and physiological responses of astrocytes toward induced spinal cord injuries. Specifically, newly generated astrocytes in glial scar borders after injury present an elongated morphology with extensive domain overlaps and interdigitation, whereas reactive astrocytes derived from matured astroglia distant to the injury would retain their 'tile' domain architecture and stellate shape, with minimal contact of their processes. Moreover, signal transducer and activator of transcription 3 is critically involved in the formation of astroglial scar, which could affect the process of interdigitation.[48]

Reactive astrocytes exert both beneficial and detrimental effects, initially protecting and enhancing neuronal survival from oxidative stress, inflammation and hypoxic/ischemic episodes. Ablation of astrocyte reactivity has been shown to increase leukocyte trafficking into the CNS, BBB repair deficiency with vasogenic brain edema production, demyelination and neuronal degeneration.[49,50] Conversely, prolonged astrocytic activation can inhibit axonal regeneration by inducing the production of persisting mature glial scars; furthermore, reactive astrocytes can release proinflammatory and cytotoxic cytokines.[51,52] The generation of ROS, nitric oxide (NO) and Aβ toxicities have been hypothesized to be facilitated by astrocytes.[53,54]

Astrocytic activation is a complicated process with highly variable outcomes. Recent evidence proposes the existence of different subtypes of reactive astrocytes that are dependent on the type of stimuli received. For example, differences in gene expression profiling were elicited between astrocytes subjected to ischemic stroke and neuroinflammation. Middle cerebral artery occlusion generated reactive astrocytes with upregulated levels of thrombospondins and growth factors, which are important for synaptic repair, hence indicating a neuroprotective role. Lipopolysaccharide injection, however, would induce astrocytic activation with the upregulation of classical complement cascade proteins, leading to synaptic loss and neuronal degeneration.[55] Furthermore, it appears that reactive astrocytes, as a single entity, can have different reactions to the same stimuli, thereby generating markedly heterogeneous responses. It has been reported that the effects of inflammatory mediators on individual reactive astrocyte can generate fluctuating expression levels of GFAP, interleukin 6 (IL-6) and chemokine ligand 7.[56]

### Astrocytes and ischemia

Evidence shows that chronic ischemia contributes to a series of pathophysiological conditions in the development of neurodegenerative diseases, including AD. Vascular damage, as a result of inflammation and oxidative stress, would lead to CBF interruption and subsequent oxygen and energy deprivations, thereby causing the eventual neuronal demise. In addition, increase in Aβ deposition and τ-phosphorylation occurrences also contribute to neurodegeneration.[20,57–59]

Astrocytes, being the principal glycogen storage in the CNS, are more resilient in surviving energy deprivation in anaerobic conditions, with the ability to increase the expressions of antioxidant enzymes such as glutathione and superoxide dismutase, and anti-apoptotic protein levels such as Bcl-2.[60] Moreover, astrocytes undergo astrogliosis within the injured area, enhancing neuronal plasticity and regenerative purposes.[61] Reactive astrocytes are capable of supplying energy to dying neurons, preventing inflammation from spreading, restoring normal pH conditions, uptaking excess glutamate, repairing tissue damage, and upregulating neurotrophic and growth factors such as transforming growth factor-β1 and endothelin-1 (ET-1).[62,63] Under a more severe ischemic insult, however, in addition to astrocytic apoptosis and autophagy, a series of pathogenic scenarios would occur, resulting in neuronal regeneration inhibition and ultimately to neuronal demise.[32,64–67] Among these events include the release of excitotoxic glutamate, production of proinflammatory cytokines and chemokines, formation of brain edema by the upregulation of aquaporin 4 water channels and the production of C-terminal of amyloid precursor protein (APP) that contributes to the eventual increase in total Aβ burden.

### Other glial cells in ischemia

Microglia constitutes the CNS-resident immune cells. They are responsible for immune surveillance, elimination of dead cells and synaptic pruning during neuronal development. During an injury, they rapidly surround the affected site and become activated.[68] In cerebral ischemia, neuronal glutamate release would trigger microglial activation.[69] Activated microglia release several neurotrophic factors—BDNF, nerve growth factor—and help to restore homeostasis in the post-ischemic brain; however, in the presence of persisting insult, activated microglia would participate in neuroinflammatory response by releasing a number of neurotoxic molecules such as free oxygen radicals, NO, tumor necrosis factor-α (TNF-α) and IL-1β, and stimulate astrogliosis.[70] In both activated microglia and astrocytes, superoxide and NO can react to produce peroxynitrite, which is a highly toxic radical species that can cause significant oxidative injury. Moreover, astroglial inducible NO synthase-mediated NO release during ischemic condition would compromise mitochondrial electron transfer process.[71,72]

Oligodendrocytes are the myelin-producer cells in the CNS, enveloping the axon in a thick layer of myelin. Myelination acts as an insulator to accelerate action potential, thereby allowing faster communication. Demyelination often occurs after an ischemic episode. Unlike astrocytes, oligodendrocytes are particularly susceptible to oxidative stress and energy deprivation.[70]

## AMYLOIDOGENESIS THROUGH AN ASTROCYTIC PERSPECTIVE

### Pathophysiology of Aβ peptide

Aβ is derived from the APP, which is an integral membrane protein that has been localized at the Golgi apparatus, endoplasmic reticulum, endosomes, lysosomes, mitochondria and plasma membranes.[73] In order to produce Aβ, APP is cleaved by β-secretase—also known as β-site APP cleaving enzyme 1 (BACE1)—at its N-terminal, followed by γ-secretase at its C-terminal, generating Aβ fragments of 40, 42 or 43 residues.[74,75] Alternatively, APP can be cleaved by α-secretase instead of BACE1, thereby precluding the formation of Aβ.[76]

It has long been thought that fibrillar Aβ found in the extracellular space as insoluble aggregated depositions are responsible for the observed neurotoxicity in AD. However, mounting data over the past two decades are supporting the notion that the soluble oligomeric Aβ is as neurotoxic and is found to be significantly increased in the AD brains.[77,78] Comparing between the oligomers and fibrils, oligomers can induce a different repertory of proinflammatory molecules secreted by activated microglia and jeopardize neuronal viability by tenfold greater than fibrils.[79,80]

In a number of studies, amyloid oligomers located intracellularly in neurons and astrocytes have been reported to disrupt $Ca^{2+}$ homeostasis, leading to neurodegeneration.[53,81–83] Several research groups suggest that the mechanism through which this is accomplished involves the ability of intracellular Aβ to permeabilize membranes and to activate the *N*-methyl-D-aspartate receptors.[84,85] These pathological events generate intracellular cytosolic $Ca^{2+}$ elevation, thereby triggering mitochondrial dysfunction, nicotinamide adenine dinucleotide phosphate oxidase activation, ROS generation, glutathione depletion, CBF dysregulation and excessive neurotoxic glutamate accumulation in the extracellular space. These mechanisms lead to subsequent oxidative injury and synaptic dysfunction.[86,87]

Recent studies have reported the existence of 'pore-like structures' of Aβ formed by the circularization of amyloid prefibrillar oligomers subunits, referred to as amyloid annular protofibrils (APFs), localized on plasma membranes, cellular vesicles in neurons and inside activated astrocytes.[88,89] It has been suggested that the pathogenicity of prefibrillar oligomers reside in their ability to permeabilize the membrane, adhering to it and forming a calcium-permeable channel, resulting in the disruption of intracellular $Ca^{2+}$ homeostasis.[90] In congruence with the APF-proposed pathogenicity hypothesis, other protofibrillar structure molecules such as α-synuclein in Parkinson's disease, ABri amyloid subunit in the familial British dementia and islet amyloid polypeptide in type II diabetes have been implicated to exert the same membrane permeabilization effect.[91–93]

Amyloid plaque formation is the consequence of fibril accumulation in the brain extracellular space. Fibrils have been commonly assumed to be derived from oligomers in a pathway involving Aβ APFs as intermediates, which lead to the belief that the existence of amyloid plaques could be used as a reliable biomarker of AD progression. However, newer evidence suggests that Aβ APFs are products of a distinct pathway that does not end in the subsequent fibrils formation.[94] This provides a possible explanation that fibrillar plaques are found in non-demented patients and emphasize the importance of Aβ APFs as the oligomeric cytotoxic conformation in AD.[95]

Aβ could also induce neurotoxicity by promoting τ-phosphorylation through the cyclin-dependent kinase 5 pathway, which is often accompanied by ROS increase, along with activations of the caspase 3, JUN kinase and nuclear factor κ-light-chain-enhancer of activated B cells (NFKB) pathways.[96,97] In oligodendrocytes, Aβ activates the neutral sphyngomyelinase–ceramide pathway, causing DNA fragmentation and mitochondrial functional impairment, resulting in oligodendrocytic apoptosis and necrosis.[98,99] Both astrocytes and microglia have phagocytic activity and can clear Aβ. On the other hand, activated microglia found in the periphery of Aβ plaques could also release pro-inflammatory cytokines, thereby increasing oxidative stress.[100,101]

### Astrocytic Aβ peptide

Accumulating data show the importance of astrocytes in Aβ pathogenesis in AD. Numerous debates have discussed whether astrocytes uptake Aβ from the extracellular space or they could synthesize Aβ *de novo*. Moreover, there is incongruent evidence

toward the activation of astrocytes by Aβ. The notion that astrocytes phagocytose Aβ from the extracellular space is supported by several studies, which demonstrated that Aβ was taken up by astrocytes for lysosomal degradation in order to maintain Aβ homeostasis.[53] Studies have shown that astrocytes clear preferentially N-terminal-truncated Aβ, and subsequently concluded that the Aβ was of neuronal origin.[74,102] Other reports indicated that astrocytes were more inclined to phagocytose oligomeric $Aβ_{1–42}$ than fibrillary $Aβ_{1–42}$, and that Aβ oligomers do activate astrocytes.[103,104] It is worthwhile to note that one of the studies used human astrocytes from AD patients, whereas the other used primary astrocytes derived from postnatal wild-type mice. The difference between species and tissue healthiness could explain the disparity of results in astrocytes responsiveness. In another study, a lower responsiveness to Aβ in human astrocytes from AD cases than in non-AD cases was demonstrated, suggesting that amyloid plaque formation in the extracellular space in AD cases is secondary to insufficient clearance of Aβ by astrocytes.[105]

APP and BACE1 overexpression in astrocytes may lead to Aβ synthesis *de novo*. Compelling studies demonstrate APP immunoreactivity in astrocytes and its subsequent upregulation in the post-ischemic brain along with Aβ deposition appearances.[106–108] It has been recently suggested that APP has a physiological role in astrocytic $Ca^{2+}$ oscillations, APP silencing leads to dysfunctional store-operated $Ca^{2+}$ channels.[109] One study reported an enhanced BACE1 production in activated astrocytes after stroke, thereby suggesting that Aβ accumulation in the extracellular space could be due to its production and release by activated astrocytes.[108] Furthermore, astrocytic BACE1 and APP expressions were found to have increased significantly upon stimulation with TNF-α and interferon-γ.[110,111] It has also been suggested that astrocytes may have a dual role in clearing and producing Aβ.[112]

The ability for astrocytes to degrade Aβ relies on the expression of proteases such as neprilysin, insulin-degrading enzyme, ET-converting enzyme, angiotensin-converting enzyme, plasminogen activators and matrix metalloproteinases.[105] In addition, secretion of apolipoprotein E (APOE) by astrocytes is suggested to be part of the neuronal repair as much as part of the Aβ clearance mechanisms. However, different isoforms of APOE are suggested to have different functions in AD, for example, APOE ε4 isoform has been reported to be a major risk factor, whereas APOE ε2 isoform exerts a protective role.[113] It is worthwhile to note that APOE has been implicated in vascular dysregulation, but much controversy still exists in this area.[114,115]

## ET-1 AND RAGE PARTICIPATION IN AN ISCHEMIC AD BRAIN

### Endothelin-1

ET-1 is a potent vasoconstrictor first characterized from the porcine aortic endothelial cells, generally recognized for its role in blood pressure regulation and inflammatory mediation.[116–118] In astrocytes, ET-1 is an important growth factor that can regulate its proliferation, secretion of neurotrophic factors, glucose uptake by the inhibition of gap junctional communication, cellular migration through upregulation of matrix metalloproteinase-9, as well as activation after brain injury via G-protein-coupled endothelin A receptor and endothelin B receptor.[119–125] ET-1 can also induce intracellular $Ca^{2+}$ concentration increase through the activation of these receptors. Endothelin A receptor and endothelin B receptor trigger phospholipase C, which can hydrolyze phosphatidylinositol 4,5-biphosphate to generate inositol 1,4,5-triphosphate ($IP_3$). The binding of $IP_3$ to its receptor in the endoplasmic reticulum induces $Ca^{2+}$ release into the cytoplasm.[126]

During ischemia, ET-1 is upregulated in both endothelial cells and astrocytes.[127] In astrocytic cultures, ET-1 shows a protective function against hypoxic-ischemic insults.[63] In an animal model, however, ET-1 overexpression due to transient middle artery occlusion is followed by increases of ROS, NO and prostaglandin levels, matrix metalloproteinase-2 expression and aquaporin 4 translocation. These changes would lead to the generation of oxidative and nitrosative stress, inflammation, BBB breakdown and other deleterious effects leading to the cerebrovascular dysfunction.[128–130] ET-1 preconditioning of neuronal cultures appear to exert a protective role from inflammation and oxidative stress.[131] Inflammation constitutes an important pathological event in ischemia. Recent evidence has reported that the additions of inflammatory mediators transforming growth factor-β1, lipopolysaccharide and interferon-γ to astrocytes can alter the expressions of endothelin A and B receptors among other G-protein-coupled receptors, resulting in the modulation of astrocytic calcium signaling.[56].

In relation to AD, it is known that ET-1 precursor 'pre-proendothelin-1' is cleaved by the ET-converting enzyme-2—present in neurons, astrocytes and microglia—that coincidentally is also one of many proteases that contribute to Aβ degradation. It has been proposed that Aβ overexpression upregulates ET-converting enzyme-2, synchronously enhance ET-1 production, which then leads to a decrease in CBF in AD owing to the ET-1-mediated vasoconstriction, thus suggesting ET-converting enzyme-2 as an important factor involved in AD pathology.[132]

In an attempt to study the relationship between cerebral ischemia and cognitive impairment in AD, one study showed that a combination of overdosed ET-1 and Aβ infusion, mimicking cerebral ischemia in an AD animal model, led to a more severe decline in cognition.[12] The overdosage of ET-1 and Aβ generated an increased proinflammatory response, Aβ deposition, neuronal loss, τ-phosphorylation and astrocytes activation. Moreover, it has also been demonstrated that influx of intracerebral Aβ through the BBB by RAGE induced the expression of proinflammatory cytokines such as IL-6 and TNF-α, along with ET-1 production in endothelial cells.[133] The increased ET-1 production led to a decrease in CBF. As ET-1 levels remain unchanged in the RAGE knockout mouse model, but upregulated in RAGE-associated Aβ transportation, it was concluded that ET-1-mediated vasoconstriction is RAGE-dependent.

### RAGE and cerebral ischemia

In cerebral ischemia, RAGE activation can trigger increased oxidative stress and inflammation.[134–136] As mentioned earlier, RAGE appears to be involved in the Aβ-ET-1 interaction at the BBB during brain ischemia. RAGE expression can trigger multiple downstream signaling cascades to induce chronic inflammation, involving the activation of NFKB.[137] RAGE was first described to bind advanced glycation end products on the cell surface of endothelial cells in hyperglycemia cases.[138] It is now known that RAGE is localized on the cell membrane as receptor to multiple ligands, such as advanced glycation end product, Aβ, a family of S100/calgranulins, β2-integrins and high mobility group box 1 protein. RAGE is found in various cells, including microglia, astrocytes, neurons and endothelial cells.[139]

Research in AD has shown that RAGE is responsible of Aβ influx from circulating plasma into the brain through the BBB.[22] RAGE overexpression in endothelial cells can cause BBB leakage by decreasing F-actin stress fibers through β-catenin increment, thus preventing membrane resealing. Without an intact BBB, the influx/efflux homeostasis of biomolecules between the blood and the brain is interrupted, leading to a cascade of pathological events contributing to neuronal demise.[140]

RAGE expression is not limited to the endothelial cells in the BBB. Studies have explored its role in neurons, microglia and astrocytes. Aβ–RAGE interaction induces mitochondrial dysfunction in cortical neurons through the activation of p38 mitogen-activated protein kinase. Furthermore, it was shown that Aβ is

internalized in cortical neurons through a RAGE-dependent pathway.[141] On astrocyte surface, the same Aβ–RAGE ligand–receptor interaction leads to extracellular signal-regulated kinases 1/2 and calcium-dependent phospholipase A2 phosphorylation, along with ROS production through a different mechanism than in cerebral endothelial cells.[142] In microglia, overexpression of RAGE causes an enhanced production of IL-1β, TNF-α and amyloid accumulation.[143] Moreover, synthesis of Aβ in neurons has been shown to be mediated by a RAGE-dependent pathway, involving the activation of the nuclear factor of activated T cell 1 (NFAT1) through $Ca^{2+}$ concentration increment, which in turn upregulated BACE1 expression after RAGE activation.[144]

In studying other factors that are influenced by RAGE expression, one study reported that the secretion of the proinflammatory cytokines IL-6 and TNF-α in astrocytes on S100B stimulation was through a RAGE-dependent manner.[145] Furthermore, both extracellular glutamate and S100B have been shown to enhance RAGE expression independently in cortical neurons, thus providing evidence of the importance of S100B—a factor enhanced and secreted on astroglial stimulation—in RAGE activation.[146]

## MECHANISM UNDERLYING Aβ PRODUCTION IN ASTROCYTES

The previous sections have discussed the potential importance of vascular lesions in the development of neurodegeneration as observed in AD. Moreover, the NVU and astrocytes are likely to have pivotal roles in the initiation of the early pathologies that lead to the eventual neuronal demise.

In attempt to elucidate one of the mechanisms leading to Aβ production in astrocytes during ischemic conditions, we hypothesize that the increase of ET-1 as a result of astrocytic hypoxic-ischemic stress, ET-1 acts in an autocrine manner to activate astrocytic responsiveness (Figure 1).[127] S100B, as a result of astrocytic reactivity, binds to its receptor RAGE in an autocrine manner. S100B–RAGE complexes are internalized to the cytosol,

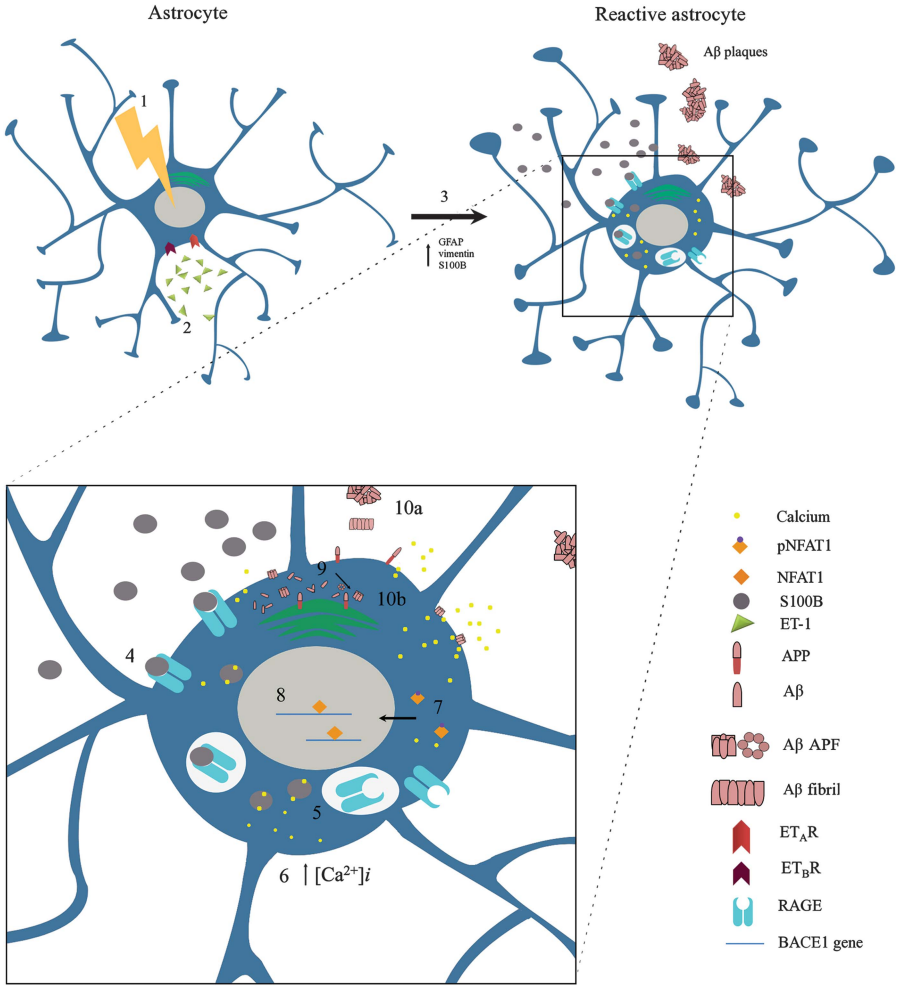


**Figure 1.** β-Amyloid (Aβ)–peptide generation in astrocytes. Under hypoxic-ischemic conditions (1), healthy astrocytes increase endothelin-1 (ET-1) production and secretion (2). ET-1 autocrine interaction with its receptors endothelin A receptor ($ET_AR$) and endothelin B receptor ($ET_BR$) activates astrocytes (3), upregulating glial fibrillary acidic protein (GFAP), vimentin and S100B production. On extracellular S100B interaction with its receptor for advanced glycation end product (RAGE), both are translocated to the cytosol by endocytosis, but RAGE is recycled to the membrane (5), whereas S100B binds to calcium, causing intracellular calcium raise, disrupting calcium homeostasis (6). On intracellular calcium increment, phosphorylated nuclear factor of activated T cell 1 (pNFAT1) is dephosphorylated and translocated to the nucleus (7), binding to β-site amyloid precursor protein (APP) cleaving enzyme (BACE1) gene promoter and activating BACE1 transcription (8). APP cleavage by BACE1 will be favored, generating more Aβ oligomers (9). Extracellular Aβ may form fibrils that will consecutively lead to dense core plaques formation (10a), whereas intracellular Aβ may form amyloid annular protofibrils (APFs) that consecutively will lead to membrane disruption through the formation of a calcium-permeable channel (10b).

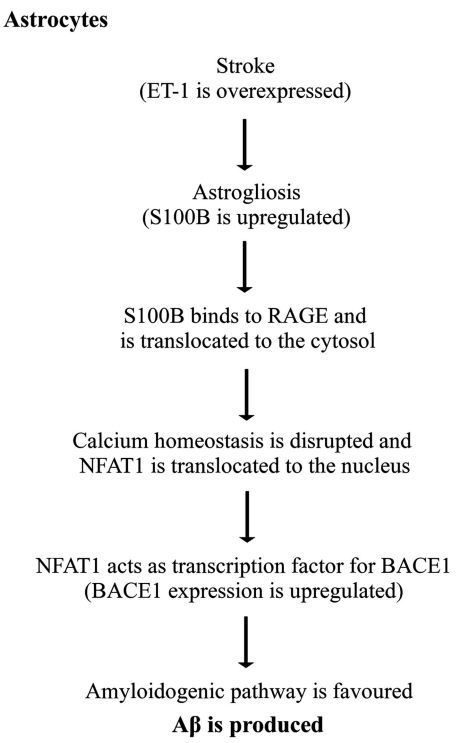


**Figure 2.** Astrocytic β-amyloid (Aβ)–peptide synthesis in stroke. After an ischemic episode, endothelin-1 (ET-1) is upregulated, inducing astrogliosis. S100B expression will be enhanced after astrocytes reactivation and will be translocated to the cytosol on its binding to receptor for advanced glycation end product (RAGE). As a result, calcium homeostasis will be disrupted by S100B interaction with calcineurin. Phosphorylated nuclear factor of activated T cell 1 (pNFAT1) will be dephosphorylated and translocated to the nucleus, where it might bind a gene promoter region of β-site amyloid precursor protein (APP) cleaving enzyme (BACE1), upregulating BACE1 expression. With more BACE1 to cleave APP, the amyloidogenic pathway is favored producing more Aβ.

but RAGE is then recycled to the membrane.[147] The sudden increment of S100B in the cytosol causes calcium homeostasis disruption, raising intracellular calcium concentrations, activating NFAT1 translocation to the nucleus. NFAT1 acts as a transcription factor for the BACE1 gene, favoring APP cleavage to Aβ production nuclear factor of activated T-cells.[144] Aβ could then go through two different pathways, forming either Aβ fibrillar plaques or Aβ APFs.[94] Under ischemic condition such as stroke, the described putative events would lead to increased Aβ production, thereby leading to the neurodegeneration observed in MixD and VaD (Figure 2).

## SUMMARY

Increasing number of studies in the literature has pointed out the significant involvement of vascular lesions in AD patients, thereby leading to the conceptualization of MixD as a distinct dementia etiology. Some researchers argue that MixD is in fact the most common form of dementia. Although the MixD concept remains unclear, it is generally accepted that cognitive decline in these cases is resulted from vascular damage in conjunction with amyloid deposition and neurofibrillary tangles formation. As the NVU represents the functional unit in maintaining cerebral vascular and transport homeostasis, the disruption of its proper functioning could be important in the neurodegenerative processes that occur in MixD and VaD. Furthermore, astrocytes, being an essential component of the NVU, form the intimate link between the endothelium and neurons. During an ischemic insult, astrogliosis acts as a 'double-edged sword' in restoring the brain microenvironment but could also lead to ROS-mediated and inflammatory damages. In relation to AD and MixD, astrocytes appear to be closely linked to Aβ pathogenicity. The interactions between RAGE, ET-1 and astrocytic activation in ischemia likely have a critical role in amyloidogenesis. In view of the high prevalence of AD/MixD and the lack of thorough understanding of AD/MixD mechanisms, further pursuit of knowledge with respect to astrogliosis and NVU dysfunction in dementia pathogenesis is highly warranted.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

1 Qiu C, De Ronchi D, Fratiglioni L. The epidemiology of the dementias: an update. *Curr Opin Psychiatry* 2007; **20**: 380–385.
2 Galimberti D, Scarpini E. Progress in Alzheimer's disease. *J Neurol* 2012; **259**: 201–211.
3 Brun A, Englund E. Regional pattern of degeneration in Alzheimer's disease: neuronal loss and histopathological grading. *Histopathology* 1981; **5**: 549–564.
4 Brun A, Liu X, Erikson C. Synapse loss and gliosis in the molecular layer of the cerebral cortex in Alzheimer's disease and in frontal lobe degeneration. *Neurodegeneration* 1995; **4**: 171–177.
5 Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun* 1984; **120**: 885–890.
6 Kosik KS, Joachim CL, Selkoe DJ. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proc Natl Acad Sci USA* 1986; **83**: 4044–4048.
7 Enciu AM, Constantinescu SN, Popescu LM, Muresanu DF, Popescu BO. Neurobiology of vascular dementia. *J Aging Res* 2011; **2011**: 401604.
8 Wallin A, Milos V, Sjogren M, Pantoni L, Erkinjuntti T. Classification and subtypes of vascular dementia. *Int Psychogeriatr* 2003; **15**(Suppl 1): 27–37.
9 Erkinjuntti T. Vascular cognitive deterioration and stroke. *Cerebrovasc Dis* 2007; **24**(Suppl 1): 189–194.
10 Lee AY. Vascular dementia. *Chonnam Med J* 2011; **47**: 66–71.
11 Zlokovic BV. Vascular disorder in Alzheimer's disease: role in pathogenesis of dementia and therapeutic targets. *Adv Drug Deliv Rev* 2002; **54**: 1553–1559.
12 Li J, Wang YJ, Zhang M, Fang CQ, Zhou HD. Cerebral ischemia aggravates cognitive impairment in a rat model of Alzheimer's disease. *Life Sci* 2011; **89**: 86–92.
13 Langa KM, Foster NL, Larson EB. Mixed dementia: emerging concepts and therapeutic implications. *JAMA* 2004; **292**: 2901–2908.
14 Zekry D, Hauw JJ, Gold G. Mixed dementia: epidemiology, diagnosis, and treatment. *J Am Geriatr Soc* 2002; **50**: 1431–1438.
15 Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 2007; **69**: 2197–2204.
16 Sheng B, Cheng LF, Law CB, Li HL, Yeung KM, Lau KK *et al.* Coexisting cerebral infarction in Alzheimer's disease is associated with fast dementia progression: applying the National Institute for Neurological Disorders and Stroke/Association Internationale pour la Recherche et l'Enseignement en Neurosciences Neuroimaging Criteria in Alzheimer's Disease with Concomitant Cerebral Infarction. *J Am Geriatr Soc* 2007; **55**: 918–922.
17 Zekry D, Gold G. Management of mixed dementia. *Drugs Aging* 2010; **27**: 715–728.
18 Fillenbaum GG, van Belle G, Morris JC, Mohs RC, Mirra SS, Davis PC *et al.* Consortium to Establish a Registry for Alzheimer's Disease (CERAD): the first twenty years. *Alzheimers Dement* 2008; **4**: 96–109.
19 Chui HC, Victoroff JI, Margolin D, Jagust W, Shankle R, Katzman R. Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. *Neurology* 1992; **42**: 473–480.
20 Jablonski M, Maciejewski R, Januszewski S, Ulamek M, Pluta R. One year follow up in ischemic brain injury and the role of Alzheimer factors. *Physiol Res* 2011; **60** (Suppl 1): S113–S119.

21 Garcia-Alloza M, Gregory J, Kuchibhotla KV, Fine S, Wei Y, Ayata C *et al.* Cerebrovascular lesions induce transient beta-amyloid deposition. *Brain* 2011; **134**: 3697–3707.
22 Bell RD, Zlokovic BV. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. *Acta Neuropathol* 2009; **118**: 103–113.
23 Iadecola C. The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. *Acta Neuropathol* 2010; **120**: 287–296.
24 Yang GY, Betz AL. Reperfusion-induced injury to the blood-brain barrier after middle cerebral artery occlusion in rats. *Stroke* 1994; **25**: 1658–1664.
25 Zhang X, Zhou K, Wang R, Cui J, Lipton SA, Liao FF *et al.* Hypoxia-inducible factor 1alpha (HIF-1alpha)-mediated hypoxia increases BACE1 expression and beta-amyloid generation. *J Biol Chem* 2007; **282**: 10873–10880.
26 Zacchigna S, Lambrechts D, Carmeliet P. Neurovascular signalling defects in neurodegeneration. *Nat Rev Neurosci* 2008; **9**: 169–181.
27 Schuff N, Matsumoto S, Kmiecik J, Studholme C, Du A, Ezekiel F *et al.* Cerebral blood flow in ischemic vascular dementia and Alzheimer's disease, measured by arterial spin-labeling magnetic resonance imaging. *Alzheimers Dement* 2009; **5**: 454–462.
28 Cai Z, Zhao B, Ratka A. Oxidative stress and beta-amyloid protein in Alzheimer's disease. *Neuromolecular Med* 2011; **13**: 223–250.
29 Verkhratsky A, Rodriguez JJ, Parpura V. Astroglia in neurological diseases. *Future Neurol* 2013; **8**: 149–158.
30 Bushong EA, Martone ME, Jones YZ, Ellisman MH. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J Neurosci* 2002; **22**: 183–192.
31 Ogata K, Kosaka T. Structural and quantitative analysis of astrocytes in the mouse hippocampus. *Neuroscience* 2002; **113**: 221–233.
32 Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol* 2010; **119**: 7–35.
33 Miller RH, Raff MC. Fibrous and protoplasmic astrocytes are biochemically and developmentally distinct. *J Neurosci* 1984; **4**: 585–592.
34 Emsley JG, Macklis JD. Astroglial heterogeneity closely reflects the neuronal-defined anatomy of the adult murine CNS. *Neuron Glia Biol* 2006; **2**: 175–186.
35 Clarke LE, Barres BA. Emerging roles of astrocytes in neural circuit development. *Nat Rev Neurosci* 2013; **14**: 311–321.
36 Oberheim NA, Goldman SA, Nedergaard M.. Heterogeneity of astrocytic form and function. *Methods Mol Biol* 2012; **814**: 23–45.
37 Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci* 2009; **32**: 421–431.
38 Takano T, Tian GF, Peng W, Lou N, Libionka w, Han X *et al.* Astrocyte-mediated control of cerebral blood flow. *Nat Neurosci* 2006; **9**: 260–267.
39 Filosa JA, Bonev AD, Straub SV, Meredith AL, Wilkerson MK, Aldrich RW *et al.* Local potassium signaling couples neuronal activity to vasodilation in the brain. *Nat Neurosci* 2006; **9**: 1397–1403.
40 Santello M, Volterra A. Synaptic modulation by astrocytes via $Ca^{2+}$-dependent glutamate release. *Neuroscience* 2009; **158**: 253–259.
41 Anderson CM, Swanson RA. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* 2000; **32**: 1–14.
42 Abbott NJ. Astrocyte-endothelial interactions and blood-brain barrier permeability. *J Anat* 2002; **200**: 629–638.
43 Strohschein S, Hüttmann K, Gabriel S, Binder DK, Heinemann U, Stainhäuser C. Impact of aquaporin-4 channels on $K^+$ buffering and gap junction coupling in the hippocampus. *Glia* 2011; **59**: 973–980.
44 Malhotra SK, Svensson M, Aldskogius H, Bhatnagar R, Das GD, Shnitka TK. Diversity among reactive astrocytes: proximal reactive astrocytes in lacerated spinal cord preferentially react with monoclonal antibody J1-31. *Brain Res Bull* 1993; **30**: 395–404.
45 Pekny M, Pekna M. Astrocyte intermediate filaments in CNS pathologies and regeneration. *J Pathol* 2004; **204**: 428–437.
46 Wilhelmsson U, Bushong EA, Price DL, Smarr BL, Phung V, Terada M *et al.* Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. *Proc Natl Acad Sci USA* 2006; **103**: 17513–17518.
47 Oberheim NA, Tian GF, Han X, Peng W, Takano T, Ransom B *et al.* Loss of astrocytic domain organization in the epileptic brain. *J Neurosci* 2008; **28**: 3264–3276.
48 Wanner IB, Anderson MA, Song B, Levine J, Fernandez A, Gray-Thompson Z *et al.* Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord injury. *J Neurosci* 2013; **33**: 12870–12886.
49 Bush TG, Puvanachandra N, Horner CH, Polito A, Ostenfeld T, Svendsen CN *et al.* Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. *Neuron* 1999; **23**: 297–308.
50 Faulkner JR, Herrmann JE, Woo MJ, Tansey KE, Doan NB, Sofroniew MV. Reactive astrocytes protect tissue and preserve function after spinal cord injury. *J Neurosci* 2004; **24**: 2143–2155.
51 Myer DJ, Gurkoff GG, Lee SM, Hovda DA, Sofroniew MV. Essential protective roles of reactive astrocytes in traumatic brain injury. *Brain* 2006; **129**: 2761–2772.
52 Zhang D, Hu X, Qian L, O'Callaghan JP, Hong JS. Astrogliosis in CNS pathologies: is there a role for microglia? *Mol Neurobiol* 2010; **41**: 232–241.
53 Funato H, Yoshimura M, Yamazaki T, Saido TC, Ito Y, Yokofujita J *et al.* Astrocytes containing amyloid beta-protein (Abeta)-positive granules are associated with Abeta40-positive diffuse plaques in the aged human brain. *Am J Pathol* 1998; **152**: 983–992.
54 Chen Y, Vartiainen NE, Ying W, Chan PH, Koistinaho J, Swanson RA. Astrocytes protect neurons from nitric oxide toxicity by a glutathione-dependent mechanism. *J Neurochem* 2001; **77**: 1601–1610.
55 Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG *et al.* Genomic analysis of reactive astrogliosis. *J Neurosci* 2012; **32**: 6391–6410.
56 Hamby ME, Coppola G, Ao Y, Geschwind DH, Khakh BS, Sofroniew MW. Inflammatory mediators alter the astrocyte transcriptome and calcium signaling elicited by multiple G-protein-coupled receptors. *J Neurosci* 2012; **32**: 14489–14510.
57 Koistinaho M, Koistinaho J. Interactions between Alzheimer's disease and cerebral ischemia--focus on inflammation. *Brain Res Brain Res Rev* 2005; **48**: 240–250.
58 Pluta R, Ulamek M, Jablonski M. Alzheimer's mechanisms in ischemic brain degeneration. *Anat Rec (Hoboken)* 2009; **292**: 1863–1881.
59 Bomboi G, Castello L, Cosentino F, Giubilei F, Orzi F, Volpe M. Alzheimer's disease and endothelial dysfunction. *Neurol Sci* 2010; **31**: 1–8.
60 Panickar KS, Norenberg MD. Astrocytes in cerebral ischemic injury: morphological and general considerations. *Glia* 2005; **50**: 287–298.
61 Barreto GE, Gonzalez J, Torres Y, Morales L. Astrocytic-neuronal crosstalk: implications for neuroprotection from brain injury. *Neurosci Res* 2011; **71**: 107–113.
62 Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. *Glia* 2005; **50**: 427–434.
63 Ho MC, Lo AC, Kurihara H, Yu AC, Chung SS, Chung SK. Endothelin-1 protects astrocytes from hypoxic/ischemic injury. *FASEB J* 2001; **15**: 618–626.
64 Yu AC, Wong HK, Yung HW, Lau LT. Ischemia-induced apoptosis in primary cultures of astrocytes. *Glia* 2001; **35**: 121–130.
65 Pluta R. Astroglial expression of the beta-amyloid in ischemia-reperfusion brain injury. *Ann N Y Acad Sci* 2002; **977**: 102–108.
66 Li L, Lundkvist A, Andersson D, Wilhelmsson U, Nagai N, Pardo AC *et al.* Protective role of reactive astrocytes in brain ischemia. *J Cereb Blood Flow Metab* 2008; **28**: 468–481.
67 Xu M, Zhang HL. Death and survival of neuronal and astrocytic cells in ischemic brain injury: a role of autophagy. *Acta Pharmacol Sin* 2011; **32**: 1089–1099.
68 Barres BA. The mystery and magic of glia: a perspective on their roles in health and disease. *Neuron* 2008; **60**: 430–440.
69 Kaushal V, Schlichter LC. Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke penumbra. *J Neurosci* 2008; **28**: 2221–2230.
70 Annunziato L, Boscia F, Pignataro G. Ionic transporter activity in astrocytes, microglia, and oligodendrocytes during brain ischemia. *J Cereb Blood Flow Metab* 2013; **33**: 969–982.
71 Brown GC, Neher JJ. Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. *Mol Neurobiol* 2010; **41**: 242–247.
72 Schubert P, Ogata T, Marchini C, Ferroni S. Glia-related pathomechanisms in Alzheimer's disease: a therapeutic target? *Mech Ageing Dev* 2001; **123**: 47–57.
73 Bi X. Alzheimer disease: update on basic mechanisms. *J Am Osteopath Assoc* 2010; **110**: S3–S9.
74 Thal DR, Schultz C, Dehghani F, Yamaguchi H, Braak H, Braak E. Amyloid beta-protein (Abeta)-containing astrocytes are located preferentially near N-terminal-truncated Abeta deposits in the human entorhinal cortex. *Acta Neuropathol* 2000; **100**: 608–617.
75 Mukherjee A, Hersh LB. Regulation of amyloid beta-peptide levels by enzymatic degradation. *J Alzheimers Dis* 2002; **4**: 341–348.
76 Evin G, Sernee MF, Masters CL. Inhibition of gamma-secretase as a therapeutic intervention for Alzheimer's disease: prospects, limitations and strategies. *CNS Drugs* 2006; **20**: 351–372.
77 Kuo YM, Emmerling MR, Vigo-Pelfrey C, Kasunic TC, Kirpatrick JB, Murdoch GH *et al.* Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. *J Biol Chem* 1996; **271**: 4077–4081.
78 Roher AE, Chaney MO, Kuo YM, Webster SD, Stine WB, Haverkamp LJ *et al.* Morphology and toxicity of Abeta-(1-42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. *J Biol Chem* 1996; **271**: 20631–20635.
79 Sondag CM, Dhawan G, Combs CK. Beta amyloid oligomers and fibrils stimulate differential activation of primary microglia. *J Neuroinflammation* 2009; **6**: 1.

80 Dahlgren KN, Manelli AM, Stine WB Jr, Baker LK, Krafft GA, LaDu MJ. Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem* 2002; **277**: 32046–32053.
81 Glabe CG, Kayed R. Common structure and toxic function of amyloid oligomers implies a common mechanism of pathogenesis. *Neurology* 2006; **66**: S74–S78.
82 Itkin A, Dupres V, Dufrêne YF, Bechninger B, Ruysschaert JM, Raussens V. Calcium ions promote formation of amyloid beta-peptide (1-40) oligomers causally implicated in neuronal toxicity of Alzheimer's disease. *PLoS One* 2011; **6**: e18250.
83 Walsh DM, Tseng BP, Rydel RE, Podlisny MB, Selkoe DJ. The oligomerization of amyloid beta-protein begins intracellularly in cells derived from human brain. *Biochemistry* 2000; **39**: 10831–10839.
84 Demuro A, Smith M, Parker I. Single-channel Ca(2-) imaging implicates Abeta1-42 amyloid pores in Alzheimer's disease pathology. *J Cell Biol* 2011; **195**: 515–524.
85 Ferreira IL, Bajouco LM, Mota SI, Auberson YP, Oliveira CR, Rego AC. Amyloid beta peptide 1-42 disturbs intracellular calcium homeostasis through activation of GluN2B-containing N-methyl-d-aspartate receptors in cortical cultures. *Cell Calcium* 2012; **51**: 95–106.
86 Abramov AY, Canevari L, Duchen MR. Calcium signals induced by amyloid beta peptide and their consequences in neurons and astrocytes in culture. *Biochim Biophys Acta* 2004; **1742**: 81–87.
87 Bambrick L, Kristian T, Fiskum G.. Astrocyte mitochondrial mechanisms of ischemic brain injury and neuroprotection. *Neurochem Res* 2004; **29**: 601–608.
88 Kokubo H, Kayed R, Glabe CG, Staufenbiel M, Saido TC, Iwata N *et al.* Amyloid Beta annular protofibrils in cell processes and synapses accumulate with aging and Alzheimer-associated genetic modification. *Int J Alzheimers Dis* 2009; **2009**: 1–7.
89 Lasagna-Reeves CA, Kayed R. Astrocytes contain amyloid-beta annular protofibrils in Alzheimer's disease brains. *FEBS Lett* 2011; **585**: 3052–3057.
90 Kayed R, Pensalfini A, Margol L, Sokolov Y, Sarsoza F, Head E *et al.* Annular protofibrils are a structurally and functionally distinct type of amyloid oligomer. *J Biol Chem* 2009; **284**: 4230–4237.
91 Volles MJ, Lee SJ, Rochet JC, Shtilerman MD, Ding TT, Kessler JC *et al.* Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry* 2001; **40**: 7812–7819.
92 Srinivasan R, Marchant RE, Zagorski MG.. ABri peptide associated with familial British dementia forms annular and ring-like protofibrillar structures. *Amyloid* 2004; **11**: 10–13.
93 Chen M, Zhang S, Liu Q, Liu P, Busuttil K, Wang C *et al.* An investigation into the formation of annular aggregates of human islet amyloid polypeptide on tantalum oxide surfaces. *Chemistry* 2012; **18**: 2493–2497.
94 Lasagna-Reeves CA, Glabe CG, Kayed R. Amyloid-beta annular protofibrils evade fibrillar fate in Alzheimer disease brain. *J Biol Chem* 2011; **286**: 22122–22130.
95 Sojkova J, Zhou Y, An Y, Kraut MA, Ferrucci L, Wong DF *et al.* Longitudinal patterns of beta-amyloid deposition in nondemented older adults. *Arch Neurol* 2011; **68**: 644–649.
96 Liu T, Perry G, Chan HW, Verdile G, Martins RN, Smith MA *et al.* Amyloid-beta-induced toxicity of primary neurons is dependent upon differentiation-associated increases in tau and cyclin-dependent kinase 5 expression. *J Neurochem* 2004; **88**: 554–563.
97 Ebenezer PJ, Weidner AM, LeVine H 3rd, Markesbery WR, Murphy MP, Zhang L *et al.* Neuron specific toxicity of oligomeric amyloid-beta: role for JUN-kinase and oxidative stress. *J Alzheimers Dis* 2010; **22**: 839–848.
98 Lee JT, Xu J, Lee JM, Ku G, Han X, Yang DI *et al.* Amyloid-beta peptide induces oligodendrocyte death by activating the neutral sphingomyelinase-ceramide pathway. *J Cell Biol* 2004; **164**: 123–131.
99 Xu J, Chen S, Ahmed SH, Chen H, Ku G, Goldberg MP *et al.* Amyloid-beta peptides are cytotoxic to oligodendrocytes. *J Neurosci* 2001; **21**: RC118.
100 Kitazawa M, Yamasaki TR, LaFerla FM. Microglia as a potential bridge between the amyloid beta-peptide and tau. *Ann N Y Acad Sci* 2004; **1035**: 85–103.
101 Cornejo F, von Bernhardi R. Role of scavenger receptors in glia-mediated neuroinflammatory response associated with Alzheimer's disease. *Mediators Inflamm* 2013; **2013**: 895651.
102 Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang HY. Astrocytes accumulate A beta 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. *Brain Res* 2003; **971**: 197–209.
103 Nielsen HM, Mulder SD, Beliën JA, Musters RJ, Eikelenboom P, Veerhuis R. Astrocytic A beta 1-42 uptake is determined by A beta-aggregation state and the presence of amyloid-associated proteins. *Glia* 2010; **58**: 1235–1246.
104 Hou L, Liu Y, Wang X, Ma H, He J, Zhang Y *et al.* The effects of amyloid-beta42 oligomer on the proliferation and activation of astrocytes *in vitro*. *In Vitro Cell Dev Biol Anim* 2011; **47**: 573–580.
105 Mulder SD, Veerhuis R, Blankenstein MA, Nielsen HM. The effect of amyloid associated proteins on the expression of genes involved in amyloid-beta clearance by adult human astrocytes. *Exp Neurol* 2012; **233**: 373–379.
106 Banati RB, Gehrmann J, Wiessner C, Hossmann KA, Kreutzberg GW. Glial expression of the beta-amyloid precursor protein (APP) in global ischemia. *J Cereb Blood Flow Metab* 1995; **15**: 647–654.
107 Popa-Wagner A, Schroder E, Walker LC, Kessler C. beta-Amyloid precursor protein and ss-amyloid peptide immunoreactivity in the rat brain after middle cerebral artery occlusion: effect of age. *Stroke* 1998; **29**: 2196–2202.
108 Nihashi T, Inao S, Kajita Y, Kawai T, Sugimoto T, Niwa M *et al.* Expression and distribution of beta amyloid precursor protein and beta amyloid peptide in reactive astrocytes after transient middle cerebral artery occlusion. *Acta Neurochir (Wien)* 2001; **143**: 287–295.
109 Linde CI, Baryshnikov SG, Mazzocco-Spezzia A, Golovina VA. Dysregulation of $Ca^{2+}$ signaling in astrocytes from mice lacking amyloid precursor protein. *Am J Physiol Cell Physiol* 2011; **300**: C1502–C1512.
110 Hartlage-Rubsamen M, Zeitschel U, Apelt J, Gärtner U, Franke H, Stahl T *et al.* Astrocytic expression of the Alzheimer's disease beta-secretase (BACE1) is stimulus-dependent. *Glia* 2003; **41**: 169–179.
111 Zhao J, O'Connor T, Vassar R. The contribution of activated astrocytes to Abeta production: implications for Alzheimer's disease pathogenesis. *J Neuroinflammation* 2011; **8**: 150.
112 Rossner S, Lange-Dohna C, Zeitschel U, Perez-Polo JR. Alzheimer's disease beta-secretase BACE1 is not a neuron-specific enzyme. *J Neurochem* 2005; **92**: 226–234.
113 Laws SM, Hone E, Gandy S, Martins RN. Expanding the association between the APOE gene and the risk of Alzheimer's disease: possible roles for APOE promoter polymorphisms and alterations in APOE transcription. *J Neurochem* 2003; **84**: 1215–1236.
114 Orsitto G, Seripa D, Panza F, Franceschi M, Cascavilla L, Placentino G *et al.* Apolipoprotein E genotypes in hospitalized elderly patients with vascular dementia. *Dement Geriatr Cogn Disord* 2007; **23**: 327–333.
115 Qi JP, Wu H, Yang Y, Wang DD, Chen YX, Gu YH *et al.* Cerebral ischemia and Alzheimer's disease: the expression of amyloid-beta and apolipoprotein E in human hippocampus. *J Alzheimers Dis* 2007; **12**: 335–341.
116 Yoshimoto S, Ishizaki Y, Mori A, Sasaki T, Takakura K, Murota S. The role of cerebral microvessel endothelium in regulation of cerebral blood flow through production of endothelin-1. *J Cardiovasc Pharmacol* 1991; **17**(Suppl 7): S260–S263.
117 de Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD *et al.* Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* 1988; **85**: 9797–9800.
118 Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y *et al.* A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; **332**: 411–415.
119 Hama H, Kasuya Y, Sakurai T, Yamada G, Suzuki N, Masaki T *et al.* Role of endothelin-1 in astrocyte responses after acute brain damage. *J Neurosci Res* 1997; **47**: 590–602.
120 Hasselblatt M, Kamrowski-Kruck H, Jensen N, Schilling L, Kratzin H, Sirén AL *et al.* ETA and ETB receptor antagonists synergistically increase extracellular endothelin-1 levels in primary rat astrocyte cultures. *Brain Res* 1998; **785**: 253–261.
121 Cazaubon S, Chaverot N, Romero IA, Girault J-A, Adamson P, Strosberg AD *et al.* Growth factor activity of endothelin-1 in primary astrocytes mediated by adhesion-dependent and -independent pathways. *J Neurosci* 1997; **17**: 6203–6212.
122 Gadea A, Schinelli S, Gallo V. Endothelin-1 regulates astrocyte proliferation and reactive gliosis via a JNK/c-Jun signaling pathway. *J Neurosci* 2008; **28**: 2394–2408.
123 Koyama Y, Baba A, Matsuda T. Endothelins stimulate the expression of neurotrophin-3 in rat brain and rat cultured astrocytes. *Neuroscience* 2005; **136**: 425–433.
124 Tabernero A, Jimenez C, Velasco A, Giaume C, Medina JM. The enhancement of glucose uptake caused by the collapse of gap junction communication is due to an increase in astrocyte proliferation. *J Neurochem* 2001; **78**: 890–898.
125 Wang HH, Hsieh HL, Wu CY, Yang CM. Endothelin-1 enhances cell migration via matrix metalloproteinase-9 up-regulation in brain astrocytes. *J Neurochem* 2010; **113**: 1133–1149.
126 Tykocki NR, Watts SW. The interdependence of endothelin-1 and calcium: a review. *Clin Sci (Lond)* 2010; **119**: 361–372.
127 Jiang MH, Höög A, Ma KC, Nie XJ, Olsson Y, Zhang WW. Endothelin-1-like immunoreactivity is expressed in human reactive astrocytes. *Neuroreport* 1993; **4**: 935–937.
128 Lo AC, Chen AY, Hung VK, Yaw LP, Fung MK, Ho MC *et al.* Endothelin-1 overexpression leads to further water accumulation and brain edema after middle cerebral artery occlusion via aquaporin 4 expression in astrocytic end-feet. *J Cereb Blood Flow Metab* 2005; **25**: 998–1011.

129 Leung JW, Chung SS, Chung SK. Endothelial endothelin-1 over-expression using receptor tyrosine kinase tie-1 promoter leads to more severe vascular permeability and blood brain barrier breakdown after transient middle cerebral artery occlusion. *Brain Res* 2009; **1266**: 121–129.
130 Filipovich T, Fleisher-Berkovich S. Regulation of glial inflammatory mediators synthesis: possible role of endothelins. *Peptides* 2008; **29**: 2250–2256.
131 Luo J, Grammas P. Endothelin-1 is elevated in Alzheimer's disease brain microvessels and is neuroprotective. *J Alzheimers Dis* 2010; **21**: 887–896.
132 Palmer JC, Baig S, Kehoe PG, Love S. Endothelin-converting enzyme-2 is increased in Alzheimer's disease and up-regulated by Abeta. *Am J Pathol* 2009; **175**: 262–270.
133 Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E *et al.* RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. *Nat Med* 2003; **9**: 907–913.
134 Kamide T, Kitao Y, Takeishi T, Okada A, Mohri H, Schmidt AM *et al.* RAGE mediates vascular injury and inflammation after global cerebral ischemia. *Neurochem Int* 2012; **60**: 220–228.
135 Muhammad S, Barakat W, Stoyanov S, Murikinati S, Yang H, Tracey KJ *et al.* The HMGB1 receptor RAGE mediates ischemic brain damage. *J Neurosci* 2008; **28**: 12023–12031.
136 Zhai DX, Kong QF, Xu WS, Bai SS, Peng HS, Zhao K *et al.* RAGE expression is up-regulated in human cerebral ischemia and pMCAO rats. *Neurosci Lett* 2008; **445**: 117–121.
137 Taguchi A. Vascular factors in diabetes and Alzheimer's disease. *J Alzheimers Dis* 2009; **16**: 859–864.
138 Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC *et al.* Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 1992; **267**: 14998–15004.
139 Han SH, Kim YH, Mook-Jung I. RAGE: the beneficial and deleterious effects by diverse mechanisms of actions. *Mol Cells* 2011; **31**: 91–97.
140 Xiong F, Leonov S, Howard AC, Xiong S, Zhang B, Mei L *et al.* Receptor for advanced glycation end products (RAGE) prevents endothelial cell membrane resealing and regulates F-actin remodeling in a beta-catenin-dependent manner. *J Biol Chem* 2011; **286**: 35061–35070.
141 Takuma K, Fang F, Zhang W, Yan S, Fukuzaki E, Du H *et al.* RAGE-mediated signaling contributes to intraneuronal transport of amyloid-beta and neuronal dysfunction. *Proc Natl Acad Sci U S A* 2009; **106**: 20021–20026.
142 Askarova S, Yang X, Sheng W, Sun GY, Lee JC. Role of Abeta-receptor for advanced glycation endproducts interaction in oxidative stress and cytosolic phospholipase A(2) activation in astrocytes and cerebral endothelial cells. *Neuroscience* 2011; **199**: 375–385.
143 Fang F, Lue LF, Yan S, Xu H, Luddy JS, Chen D *et al.* RAGE-dependent signaling in microglia contributes to neuroinflammation, Abeta accumulation, and impaired learning/memory in a mouse model of Alzheimer's disease. *FASEB J* 2010; **24**: 1043–1055.
144 Cho HJ, Son SM, Jin SM, Hong HS, Shin DH, Kim SJ *et al.* RAGE regulates BACE1 and Abeta generation via NFAT1 activation in Alzheimer's disease animal model. *FASEB J* 2009; **23**: 2639–2649.
145 Ponath G, Schettler C, Kaestner F, Voigt B, Wentker D, Arolt V *et al.* Autocrine S100B effects on astrocytes are mediated via RAGE. *J Neuroimmunol* 2007; **184**: 214–222.
146 Villarreal A, Aviles Reyes RX, Angelo MF, Reines AG, Ramos AJ. S100B alters neuronal survival and dendrite extension via RAGE-mediated NF-kappaB signaling. *J Neurochem* 2011; **117**: 321–332.
147 Perrone L, Peluso G, Melone MA. RAGE recycles at the plasma membrane in S100B secretory vesicles and promotes Schwann cells morphological changes. *J Cell Physiol* 2008; **217**: 60–71.