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Review Article

Neurovascular function in Alzheimer's disease patients and experimental models

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The ability of the brain to locally augment glucose delivery and blood flow during neuronal activation, termed neurometabolic and neurovascular coupling, respectively, is compromised in Alzheimer's disease (AD). Since perfusion deficits may hasten clinical deterioration and have been correlated with negative treatment outcome, strategies to improve the cerebral circulation should form an integral element of AD therapeutic efforts. These efforts have yielded several experimental models, some of which constitute AD models proper, others which specifically recapture the AD cerebrovascular pathology, characterized by anatomical alterations in brain vessel structure, as well as molecular changes within vascular smooth muscle cells and endothelial cells forming the blood–brain barrier. The following paper will present the elements of AD neurovascular dysfunction and review the *in vitro* and *in vivo* model systems that have served to deepen our understanding of it. It will also critically evaluate selected groups of compounds, the FDA-approved cholinesterase inhibitors and thiazolidinediones, for their ability to correct neurovascular dysfunction in AD patients and models. These and several others are emerging as compounds with pleiotropic actions that may positively impact dysfunctional cerebrovascular, glial, and neuronal networks in AD.
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Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. It is characterized by a gradual decline in memory and cognition, which has been correlated with synaptic dysfunction and loss, and eventually to neuronal death (Querfurth and LaFerla, 2010). The disease is confirmed postmortem on detection of amyloid-β (Aβ) plaques and neurofibrillary tangles in the brain. These are respectively composed of aggregated Aβ derived from the amyloid precursor protein (APP) and of abnormally hyperphosphorylated τ, a microtubule-associated protein involved in cytoskeletal stabilization (Wang *et al*, 2007). Cytokines, chemokines, and free radicals emanating from activated glial cells recruited at the site of plaques promote an inflammatory and oxidative state (Akiyama *et al*, 2000; Butterfield *et al*, 2001). Already at the preclinical stage, the AD brain is characterized by a reduced blood supply at rest (Farkas and Luiten, 2001; Hirao *et al*, 2005; Bateman *et al*, 2006; Vicenzini *et al*, 2007; Claassen *et al*, 2009) and altered perfusion to activated areas, i.e., altered neurovascular coupling or functional hyperemia (Hock *et al*, 1997; Mentis *et al*, 1998; Rombouts *et al*, 2000; Machulda *et al*, 2003; Rosengarten *et al*, 2006). It is debated whether the latter is the result of decreased demand by diseased tissue or of a compromised cerebral vasculature that is unable to optimally increase cerebral blood flow (CBF) in activated areas. The CBF insufficiency poses a threat for homeostasis and protein synthesis underlying learning (Iadecola, 2004), and could activate hypoxia-sensitive pathways culminating in the upregulation of proteins, such as Aβ and transforming growth factor-β 1 (TGF-β1) (Krupinski

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et al, 1996; Bennett *et al*, 2000; Sun *et al*, 2006), which have been shown to be detrimental for cerebrovascular structure and function (Wyss-Coray *et al*, 1997, 2000; Iadecola *et al*, 1999; Gaertner *et al*, 2005; Tong *et al*, 2005; Han *et al*, 2008). In parallel with or as a consequence of cerebrovascular dysfunction, AD patients feature decreased basal cerebral glucose utilization (CGU), particularly in parietotemporal and posterior cingulate cortex (Langbaum *et al*, 2009), and altered neurometabolic coupling (Melrose *et al*, 2009) that would be expected to instigate a brain energy crisis. Dysfunctional neurons, astrocytes, and vascular cells that together coordinate the perfusion response are most likely at the root of impaired neurovascular and neurometabolic coupling in AD. Much of this knowledge is derived from *in vitro* and *in vivo* experimental models, and these will be presented and reviewed. Finally, we will critically evaluate two drug classes with recently demonstrated efficacy against AD neurovascular dysfunction, and evaluate their usefulness in improving clinical outcome or response to AD therapy.

Neurovascular dysfunction in Alzheimer's disease

Chronic brain hypoperfusion (Farkas and Luiten, 2001; Hirao *et al*, 2005; Bateman *et al*, 2006; Vicenzini *et al*, 2007; Claassen *et al*, 2009) and altered neurovascular coupling (Hock *et al*, 1997; Mentis *et al*, 1998; Rombouts *et al*, 2000; Machulda *et al*, 2003; Rosengarten *et al*, 2006) are prominent features of AD. Resting CBF in human is $\sim$50 ml/100 g brain per minute. In AD patients, CBF is reduced by 10% to 30% in temporoparietal, frontal, and posterior cingulate cortex, and in hippocampus relative to age-matched elderly subjects, and these decreases correlate with dementia severity (for review, see Farkas and Luiten (2001)). Further, the decreases in resting CBF have been shown to be powerful correlates of progression to AD (Hirao *et al*, 2005) and of response to treatment (Yoshida *et al*, 2007). The localized CBF increase associated with mental tasks is equally altered relative to that of age-matched control patients, being either reduced (Mentis *et al*, 1998; Machulda *et al*, 2003; Rosengarten *et al*, 2006) or simultaneously reduced in some brain areas, while increased in others, perhaps in compensation (Rombouts *et al*, 2000; Scarmeas *et al*, 2004; Drzezga *et al*, 2005; Peters *et al*, 2009). For example, CBF was significantly reduced in medial temporal lobe structures of AD patients during color picture encoding, but increased in the cerebellum (Rombouts *et al*, 2000). In Peters *et al* (2009), AD patients showed reduced CBF responses in various fronto-temporal areas during the encoding of short word lists as well as during the recognition phase of the task, but an increased response in the fusiform gyrus, a structure thought to be involved with orthographic processing. The hemodynamic changes seem to become established very early in the pathological process, as they are manifest in populations at risk of developing AD, i.e., individuals with mild cognitive impairment or those expressing the *ε4* allele of apolipoprotein E, the most consistent genetic factor linked to increased AD risk and lower age of onset (Poirier *et al*, 1993; Strittmatter *et al*, 1993). In these groups, neurovascular coupling is either impaired (Lind *et al*, 2006) or increased in a compensatory manner as in AD patients, being characterized by a larger response magnitude, and neuronal recruitment to achieve performance at the level of healthy elderly controls (Bookheimer *et al*, 2000; Yetkin *et al*, 2006; reviewed in Wermke *et al* (2008)). As shown by Bookheimer *et al* (2000), the local perfusion increase during verbal recall was greater in cognitively normal apolipoprotein E *ε4* relative to apolipoprotein E *ε3* carriers, involved a larger number of brain regions, and correlated with cognitive decline in the apolipoprotein E *ε4* subjects two years later.

The Default Mode Network

In the last decade, a new concept of brain activation has emerged, allowing a more comprehensive understanding of the altered perfusion patterns in AD patients and in susceptible populations (Raichle *et al*, 2001; Wermke *et al*, 2008). According to this view, increases in perfusion during specific cognitive tasks are accompanied by decreases in other areas not required for the task, and it is thought that the deactivation of the latter regions permits resource reallocation toward task-relevant areas. The structures that are deactivated during goal-directed activities are otherwise tonically active, functionally interconnected, and part of a synchronized resting-state network (Sorg *et al*, 2007). One of the well-studied resting-state networks is known as the default mode network, comprising the posterior cingulate/precuneus, lateral parietal cortex, and medial prefrontal cortex, a network exhibiting high activity at rest, but suspended (deactivated on positron emission tomography and functional magnetic resonance imaging studies) during challenging cognitive activities, such as memory encoding (Raichle *et al*, 2001; for review, see Sperling *et al* (2010)). Evidence suggests that the ability to deactivate resting-state networks, including the default mode network, is already impaired in mild cognitive impairment and, to a greater extent, in AD patients (Rombouts *et al*, 2000; Lustig *et al*, 2003; Drzezga *et al*, 2005; Celone *et al*, 2006) (Figure 1). It has even been postulated that detecting changes in deactivation patterns may be particularly useful in early diagnosis or have predictive value, as these patterns were able to discriminate between a subgroup of mild cognitive impairment subjects who progressed to AD and those who remained cognitively intact

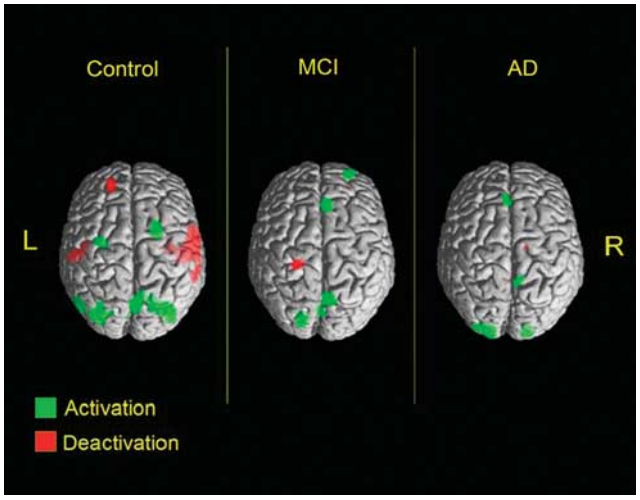

Figure 1 The concept of brain deactivation in Alzheimer's disease (AD). The ability to deactivate task-irrelevant auditory cortices during active spatial navigation was decreased in subjects with mild cognitive impairment (MCI), and to a greater extent, in those with AD. Altered regional cerebral blood flow (CBF) patterns were measured with O^{15}-positron emission tomography (PET). Reproduced from Drzezga *et al* (2005).

during a two-year follow-up period (Drzezga *et al*, 2005). The extent to which vascular versus neuronal factors contribute to these impairments is still unsettled, and complicated by the altered status of astrocytes, key mediators of the hyperemic response (Haydon and Carmignoto, 2006). Evidence supports that all components of the tripartite neurogliovascular unit (Hamel, 2006) are affected, and are likely the basis of altered neurovascular coupling patterns in AD.

The Neurogliovascular Unit

During brain activation, the control of CBF relies on the concerted action of neurons, astrocytes, and vascular cells making up a tripartite entity called the neurogliovascular unit (Iadecola, 2004; Hamel, 2006). Signals generated by neurons are ultimately transduced by cerebrovascular smooth muscle to locally adjust blood flow for the duration of the activation. The nature of the vasoactive mediators released during neurovascular coupling continues to be an area of intense investigation. It appears to depend on the type, frequency, and duration of the incoming afferent input and on its processing by the locally activated neuronal network. Vasoactive messengers released can be of neuronal, astroglial, and vascular origin (reviewed in Koehler *et al* (2009) and Cauli and Hamel (2010)). In addition, it is believed that diffuse projections from subcortical nuclei can modulate the neurovascular coupling response, either by influencing neuronal output or by directly causing vascular diameter change via contacts with intracerebral arterioles expressing relevant receptors. For example, cortical perfusion can be directly increased by stimulating the cholinergic basal forebrain (Biesold *et al*, 1989), the main source of cholinergic innervation to the neocortex. On stimulation, perivascular cortical afferents release acetylcholine (ACh) onto endothelial M_5 muscarinic ACh receptors (Elhusseiny and Hamel, 2000; Yamada *et al*, 2001). This leads to nitric oxide (NO)-mediated vasodilation and CBF increase (Vaucher and Hamel, 1995; Zhang *et al*, 1995; Kocharyan *et al*, 2008). The cholinergic system, which degenerates in AD (Whitehouse *et al*, 1981; Tong and Hamel, 1999), is central to attention (reviewed in Klinkenberg *et al* (2010)) and memory processes (reviewed in Micheau and Marighetto (2011)). Given the complex relationship among neurogliovascular components, and their interdependence in mounting an appropriate CBF response, a compromise in one or more of these elements could affect the functionality of the entire unit.

Neuronal Dysfunction

Neurons are the initiators of the functional hyperemic response. In AD, their function is threatened by pathological cascades associated with Aβ, hyperphosphorylated τ, free radicals, inflammatory factors, and perhaps still unidentified factors that lead to neurodegeneration (Querfurth and LaFerla, 2010). Presynaptic and postsynaptic markers are decreased, trophic factors, neurotransmitters, and their respective receptors are likewise reduced, as is the neuronal glucose transporter-3 (Simpson *et al*, 1994), and disturbances in axonal transport linked with neurofibrillary tangles and microtubule disarrangement are evident (Querfurth and LaFerla, 2010). The basal forebrain cholinergic system degenerates (Whitehouse *et al*, 1981; Sassin *et al*, 2000) along with noradrenaline-containing neurons of the locus coeruleus (Tomlinson *et al*, 1981), and there are decreases in levels of dopaminergic, serotoninergic, and somatostatin markers in various brain areas (Rossor *et al*, 1980; Arai *et al*, 1984). The glutamatergic system is also affected, with excessive activation of glutamate receptors resulting in neuronal Ca^{2+} overload and possibly, excitotoxic death (Hynd *et al*, 2004). Interestingly, neuronal hyperexcitability has also been documented in an AD mouse model overexpressing mutated human APP. This was measured using *in vivo* electroencephalogram in freely behaving 3- to 7-month-old animals (Palop *et al*, 2007). The ensuing compensatory inhibitory drive, consisting of GABAergic sprouting and strengthened inhibitory postsynaptic currents, resulted in profoundly altered neuronal networks (Palop *et al*, 2007). In AD, such cellular and synaptic changes may underlie the reduced capacity to disengage the default mode network during complex cognitive tasks. Namely, abnormal GABAergic function and altered ability for cross-modal inhibition

would interfere with the brain deactivation required for successful memory encoding. Evidence for a loss of inhibitory neuronal function has been reported in AD (Bélanger *et al*, 2010), leading to the hypothesis that cognitive and neurovascular dysfunction may be attributed, in part, to an imbalance between neuronal excitation and inhibition (Palop and Mucke, 2010).

Vascular Dysfunction

Profound alterations in the AD cerebral vasculature are believed to contribute to perfusion irregularities during neuronal activation (Mancardi *et al*, 1980; Roher *et al*, 1993; Vinters *et al*, 1994; Kalaria and Pax, 1995). These cerebrovascular changes may be important early factors in the aberrant hyperemic responses, as suggested by their presence in populations that are prone to develop AD, namely the elderly, subjects with chronic cardiovascular disorders (hypertension, hypercholesterolemia, and diabetes), stroke, and head trauma patients, and more so in groups exhibiting several of these factors at once (Luchsinger *et al*, 2005). The molecular mediators underlying cerebrovascular alterations in these populations and in AD are not completely elucidated, but strong evidence implicates increased levels of the Aβ and TGF-β1 peptides (Krupinski *et al*, 1996; Wyss-Coray *et al*, 1997; Grammas and Ovase, 2002; Peterson, 2005). In AD, aggregated forms of Aβ_{1-40} and Aβ_{1-42} settle within vessel walls of small to medium arteries, leading to cerebral amyloid angiopathy (CAA) (Roher *et al*, 1993). The deposition of amyloid in circumferential bands is consistent with smooth muscle cell synthesis (Roher *et al*, 1993); however, initial deposits on the abluminal side of the smooth muscle also suggest a neuronal origin. As the amyloid makes its way toward capillaries and arterioles, faulty clearance across the blood–brain barrier, and increased arterial stiffness hindering vessel pulsations that drive perivascular Aβ drainage would result in abluminal deposition (Herzig *et al*, 2006; Weller *et al*, 2008). Interestingly, CAA may also be secondary to profibrotic vascular remodeling mediated by TGF-β1 (Wyss-Coray *et al*, 1997). The TGF-β1-induced upregulation of basement membrane components, such as collagen and perlecan that bind Aβ (Castillo *et al*, 1997), could promote CAA by enhancing vessel 'adhesiveness.' With severe CAA, amyloid gradually replaces the vascular smooth muscle, impairing vasomotor function, weakening the vessel wall, and increasing the risk of cerebral hemorrhage (Christie *et al*, 2001; Boche *et al*, 2008; Han *et al*, 2008).

However, before any visible CAA, the animal literature suggests that soluble Aβ potently deregulates cerebrovascular function by activating a free radical cascade that substantially reduces vasodilator half-life and imparts oxidative damage to cerebrovascular enzymes and receptors (Price *et al*, 1997; Iadecola *et al*, 1999; Tong *et al*, 2005; Park *et al*, 2005, 2008). Soluble Aβ also potentiates constrictions to endothelin-1 (ET-1) when applied to isolated human cerebral arteries collected after rapid autopsies, seemingly via a proinflammatory cascade (Townsend *et al*, 2002; Paris *et al*, 2003). Moreover, the cholinotoxic properties of soluble Aβ (Auld *et al*, 2002; Aucoin *et al*, 2005) may be responsible for the cholinergic deafferentation of intracerebral microvessels in AD (Tong and Hamel, 1999). This perivascular denervation could explain some of the reduced hyperemic responses, particularly during tasks demanding attention, which recruit the basal forebrain. In turn, our studies in transgenic mice overproducing TGF-β1 have shown that the peptide interferes with cerebrovascular dilatory and contractile capacity by altering levels or activities of enzymes and downstream signaling cascades in the vessel wall (Tong *et al*, 2005; Tong and Hamel, 2007; Nicolakakis *et al*, 2011; Papadopoulos *et al*, 2010). Within cerebrovascular cells, several molecular changes could be directly responsible for altered hemodynamic responses. These include downregulation of the endothelial glucose transporter-1 (Simpson *et al*, 1994) and changes in endothelial receptors regulating brain Aβ influx and efflux at the level of the blood–brain barrier; upregulation of the receptor for advanced glycation end products that brings Aβ into the brain and downregulation of the low-density lipoprotein receptor-related protein that transports Aβ into the circulation (Deane *et al*, 2003). In AD vascular smooth muscle cells, increased levels of serum response factor, and myocardin, which regulate contractile protein expression, have been associated with a hypercontractile phenotype and reduced activity-driven CBF increase (Chow *et al*, 2007).

Astrocytic Dysfunction

Astrocytes are considered intermediaries in neurovascular coupling, bridging neuronal activity with perfusion through their ensheathment of neuronal synapses on one hand, and vascular appositions on the other (Haydon and Carmignoto, 2006). It can be appreciated that dysfunction in the astrocytic compartment should therefore affect the evoked hyperemic response. Indeed, in AD, several pathological cascades are induced within astrocytic cells, including expression of the β-site APP cleaving enzyme 1 that generates Aβ (Hartlage-Rübsamen *et al*, 2003). This suggests that astrocytes can transition from an amyloid clearing (Wyss-Coray *et al*, 2003) to an amyloid-generating activity with detrimental consequences for neurons and vascular cells. Moreover, there is indication that basic astrocytic communication through Ca^{2+} signaling is affected (Takano *et al*, 2007; Kuchibhotla *et al*, 2009). In a transgenic mouse model of AD, astrocytes exhibited higher resting intracellular Ca^{2+} levels viewed with *in vivo* multiphoton imaging. Astrocytes also exhibited a greater number of spontaneous Ca^{2+} oscillations that were

relatively rare in wild-type (WT) animals, and these spontaneous events were of higher amplitude and unrelated to neuronal activity, as evaluated in the presence of the sodium channel blocker tetrodotoxin (Kuchibhotla *et al*, 2009). The astrocytic hyperactivity was initiated by a single astrocyte in proximity to an Aβ plaque, and subsequently spread as an intercellular Ca^{2+} wave to distant astrocytes up to $\sim 200\,\mu$m away at speeds of $\sim 23\,\mu$m/s (Kuchibhotla *et al*, 2009). This suggested that hyperactive astrocytic networks could underlie global cortical and neurovascular coupling alterations in AD. Other studies have found that astrocytic end feet, which immediately contact the vasculature, exhibit loss of the water channel aquaporin 4 and of the inward rectifying voltage-gated potassium channel Kir4.1 (Wilcock *et al*, 2009). The latter is important for removal of rising extracellular K^+ from actively firing neurons, and for regulation of potassium signaling that can initiate diameter changes in the local microcirculation (Filosa *et al*, 2006). Finally, it is believed that chronic astroglial secretion of inflammatory mediators can induce damage in neuronal and vascular cells that respectively initiate and execute the hyperemic response. It is, however, also possible that inflammatory activity within astrocytes could interfere with neurovascular coupling via defective synthesis and release of vasomediators, such as epoxyeicosatrienoic acids (EETs) (Koehler *et al*, 2009). The combined changes would contribute to a pathological astroglial phenotype that is expected to profoundly affect neurovascular function.

Experimental models of Alzheimer's disease neurovascular dysfunction

Artery Occlusion/Stenosis

One of the earliest attempts to investigate the role of the cerebral circulation in AD pathogenesis was through artery occlusion experiments in rodents (reviewed in Farkas *et al* (2007)). Ligation of the common carotid arteries bilaterally, referred to as two-vessel occlusion, produced a significant CBF drop, astrocyte activation, and neurodegenerative changes in the brain that were associated with learning and memory impairments in behavioral tests (de la Torre *et al*, 1992; reviewed in Farkas and Luiten (2001)). Although an attractive attempt to infer causality between hypoperfusion and dementia, the two-vessel occlusion paradigm often suffered from the confounding effect of pyramidal cell damage or death and of cholinergic deficits. It also failed to mimic the gradual and chronic CBF decline that characterizes AD patients. More recent attempts to develop four-vessel occlusion in rats highlighted the importance of choosing the right type of vessel for ligation and age of the animal, as these could dictate neuropathological and behavioral outcomes (Barros *et al*, 2009). For example, occlusion of the vertebral arteries and of the common carotid arteries 1 week later resulted in hippocampal neurodegeneration, retinal lesion, and cognitive deficits in young rats 40 days after four-vessel occlusion. However, occlusion of the two vertebral and two internal carotid arteries led to a mild learning deficit in old rats only, and spared overall hippocampal cell density and retinal integrity (Barros *et al*, 2009). Accumulation of APP and its proteolytic products had been shown after ischemic and axonal injury, as a result of disrupted axonal transport (Smith *et al*, 2003). Indeed, increases in APP immunoreactivity were observed in astrocytic processes and dystrophic axons of the cortex and hippocampus of rats that had undergone repeated, reversible occlusion of the middle cerebral artery or two-vessel occlusion. However, no Aβ plaque deposition was observed (Kalaria *et al*, 1993). More recently, transient middle cerebral artery occlusion (2 hours) in rats led to focal cerebral ischemia and to the accumulation, 1 week later, of APP and Aβ into diffuse aggregates in the corpus callosum, thalamus, and cortical areas adjacent to the infarct. Nine months later, these aggregates persisted only in the thalamus, and had turned into dense, plaque-like deposits. However, they could not be stained with Congo red or Thioflavine S (van Groen *et al*, 2005). These two routinely used histological dyes detect amyloid-like proteins displaying the β-pleated sheet conformation that is typical of mature Aβ plaques. At present, the occlusion models remain good tools with which to address the role of chronic hypoperfusion in AD and in other dementias where CBF is impaired, provided confounding factors can be adequately controlled. With the recent adaptation of these techniques to mice, which involves the insertion of microcoils in the common carotids to induce stenosis rather than complete occlusion (Miki *et al*, 2009), it will be possible to evaluate additional effects of CBF reduction in transgenic AD mouse models (see Transgenic mouse models). Indeed, exposure of transgenic APP-overexpressing mice to a hypoxic environment potentiated Aβ deposition and memory deficit (Sun *et al*, 2006), suggesting an aggravating role of hypoxic events on AD pathogenesis.

Exogenous Aβ Application

It was mainly work from the group of Iadecola that drew attention to the deleterious vasoactive effects of Aβ exposure *in vivo*. Until then, most demonstrations had been obtained from *in vitro* studies on isolated human or animal cerebral arteries treated with Aβ. Those studies had shown enhanced vasoconstrictions to serotonin (5-HT) and ET-1, and impaired endothelium-dependent relaxations to ACh and bradykinin, which were attributed either to oxidative (Price *et al*, 1997; Thomas *et al*, 1997) or to proinflammatory cascades (Townsend *et al*, 2002; Paris *et al*, 2003). Topical superfusion of soluble $A\beta_{1-40}$ on the mouse neocortex yielded results that

were in line with the *in vitro* findings. It led to an attenuation of resting CBF and of the CBF increase evoked by whisker stimulation or by endothelium-dependent vasodilators, as measured by laser Doppler flowmetry (LDF) (Niwa *et al*, 2000*a*, *b*; Deane *et al*, 2003; Park *et al*, 2005; Takeda *et al*, 2009). These effects were abolished by free radical scavengers or by substitution of methionine with norleucin at residue 35 of Aβ_{1-40}, a modification that prevents free radical generation by the peptide (Niwa *et al*, 2000*b*). The impairments were also abolished by gp91ds-tat, a peptide inhibitor of NADPH oxidase, the major source of superoxide ($O_2^{\bullet -}$) in cerebral vessels. Further, no deficits were seen in mice lacking the gp91^{phox} catalytic subunit of NADPH oxidase (Park *et al*, 2005). The collective findings supported an Aβ-induced oxidative stress hypothesis implicating NADPH oxidase-derived $O_2^{\bullet -}$. At odds with this body of evidence was a study by the group of Rowan. The authors developed a method to simultaneously record electrophysiological and blood flow responses in rat CA1 after electrical stimulation of the Schaffer collateral/commissural pathway. High-frequency stimulation protocols triggered long-term potentiation and hippocampal blood flow increase, as measured with LDF. Intracerebroventricular injection of soluble Aβ_{1-40} dimers, Aβ_{1-42} or of the vasculotropic Aβ_{1-40} E22Q 30 minutes before high-frequency stimulation inhibited long-term potentiation, but had no effect on simultaneously recorded hyperemia or resting blood flow. In addition, a dose of Aβ_{1-42} capable of lowering baseline synaptic transmission by 25% did not alter baseline perfusion in the dorsal hippocampus (Hu *et al*, 2008). These findings challenged the view that soluble Aβ could initiate vascular dysfunction at concentrations inhibiting neuronal function. It also suggested that vascular deficits may act as secondary aggravating factors to the Aβ-induced neuronal pathology.

Transgenic Mouse Models

The advent of transgenic mice provided new opportunities to study the mechanisms of AD neurovascular dysfunction. Mice overexpressing mutated human APP or the Aβ-generating presenilin associated with rare early-onset AD exhibited AD neuropathology, cerebrovascular dysfunction, and memory impairments. This was especially true for mice harboring mutated human APP alone or combined with mutated human presenilin, whereas overexpression of the WT proteins in the first-generation transgenic models mostly failed to produce Aβ deposits (Mucke *et al*, 2000; reviewed in Dodart and May (2005)). Much progress has been afforded by transgenic research; however, the disappointing clinical trials that have derived from it (Abbott, 2008) remind us that the challenge remains to determine the relevance of transgenic mouse phenotypes to the human disease. Study interpretations should take into account differences between mouse and human physiology, and the potentially unknown alterations in physiology and behavior that may be initiated by insertion of human genes into the mouse genome. Other concerns include the difference between protein overexpression and natural protein upregulation or downregulation, as well as the importance of protein expression in a cell- and isoform-specific manner. Case in point is that neurons abundantly express APP$_{695}$, whereas some APP models have been engineered to overexpress, in neurons, an alternatively spliced minigene encoding all three human APP isoforms, APP$_{751,\ 770,\ 695}$ (Games *et al*, 1995; Mucke *et al*, 2000). With these caveats in mind, we highlight the following transgenic models that have greatly contributed to investigations of AD neurovascular dysfunction and to the development of therapies.

Amyloid precursor protein mice: Transgenic mice overexpressing mutated human APP with or without presenilin overproduce Aβ and develop cerebrovascular dysfunction as an early feature, often before several other neuropathological and behavioral AD symptoms (Iadecola *et al*, 1999; Niwa *et al*, 2000*a*, 2002*a*, *b*; Park *et al*, 2005; Tong *et al*, 2005; Takeda *et al*, 2009). Young APP mice (2 to 3 months old) devoid of plaque pathology and neuronal loss display an attenuated perfusion response to endothelium-dependent vasodilators, and exaggerated CBF decrease to a constrictor, the thromboxane A_2 analog U46619 (Iadecola *et al*, 1999). In addition, APP mice feature impaired autoregulatory ability, a homeostatic mechanism that ensures constant blood flow during changes in mean arterial pressure. Animals thus lack protection against ischemia (Niwa *et al*, 2002*b*; Takeda *et al*, 2009). The CBF increase to hypercapnia and endothelium-independent vasodilators such as SNAP (S-nitroso-N-acetylpenicillamine) or SNP (sodium nitroprusside) is either maintained (Iadecola *et al*, 1999; Niwa *et al*, 2000*a*; Christie *et al*, 2001; Shin *et al*, 2007) or impaired (Park *et al*, 2005; Han *et al*, 2008) in young Tg2576 mice, suggesting variable degree of smooth muscle dysfunction. However, the response to whisker stimulation is diminished, with the reduction being commensurate to levels of soluble brain Aβ_{1-40} and Aβ_{1-42} encountered in the various APP lines tested (Tg6209, Tg2123, and Tg2576) (Niwa *et al*, 2000*a*) (Table 1). However, a firm statement about the identity of the Aβ isoform responsible for the deficits could not be made. Furthermore, it was unclear in the latter study if neuronal dysfunction had a role. Quantitative radioautographical determination of CGU showed intact resting and evoked glucose usage in Tg2123 mice (Niwa *et al*, 2000*a*), but a subsequent study showed reduced basal glucose utilization in Tg2576 mice that are derived from a different background strain and produce higher Aβ levels (Niwa *et al*, 2002*a*). Recently, Takeda *et al* (2009) adjusted the magnitude of whisker stimulation so

Table 1 Neurovascular coupling deficits during sensory stimulation in transgenic mouse models of AD or of the AD cerebrovascular pathology

Model	*Age (months)*	*Deficit (%)*	*Technique*	*Reference*
APP				
Tg6209	2–3	20[a]	LDF	Niwa *et al* (2000a)
Tg2123	2–3	47[a]	LDF	Niwa *et al* (2000a)
Tg2123	2–3	39	IAP	Niwa *et al* (2000a)
Tg2576	2–3	60[a]	LDF	Niwa *et al* (2000a)
Tg2576	2–3	48[a]	LDF	Park *et al* (2005)
Tg2576	3–4	42[a]	LDF	Park *et al* (2008)
Tg2576	8	NS	LSF	Shin *et al* (2007)
Tg2576	12–15	21[a]	LDF	Park *et al* (2008)
Tg2576	19	44[a]	LSF	Shin *et al* (2007)
J20	7.5	37	LDF	This paper
J20	12	36	LDF	Tong *et al* (2009)
J20	~15	42	LDF	Nicolakakis *et al* (2008)
APP23	3	73[a]	LDF	Takeda *et al* (2009)
APP23	6	NS	fMRI	Mueggler *et al* 2003)
APP23	13	NS	fMRI	Mueggler *et al* (2003)
APP23	25	54	fMRI	Mueggler *et al* (2003)
TGF				
T64	6–8	38	LDF	Ongali *et al* (2010)
T64	12	27*	LDF	Ongali *et al* (2010)
T64	~18	24	LDF	Nicolakakis *et al* (2011)
T64	~18	27	LDF	Papadopoulos *et al* (2010)
APP/TGF				
J20/T64	6–8	NS	LDF	Ongali *et al* (2010)
J20/T64	12	31	LDF	Ongali *et al* (2010)
J20/T64	~18	41	LDF	Ongali *et al* (2010)

AD, Alzheimer's disease; APP, amyloid precursor protein; NS, not significant; IAP, quantitative ^{14}C-labeled iodoantipyrine technique in awake mice; LDF, laser Doppler flowmetry; LSF, laser speckle flowmetry; TGF, transforming growth factor-β1.

[a]Value is an estimate obtained from manuscript graph by comparing values between transgenic and age-matched WT mice (hence standard errors are not indicated). *$P = 0.057$.

as to equalize somatosensory evoked potentials recorded at the site of CBF measurement. The authors were thus able to exclude that differences in neuronal activation accounted for the functional hyperemic deficit between 3-month-old WT and APP23 mice. Hence, the data in young transgenic APP mice support the deleterious effects of soluble Aβ on cerebrovascular function.

Interestingly, and in accord with the *in vitro* and *in vivo* superfusion data (see Exogenous Aβ application), endothelial dysfunction does not occur in young APP mice co-overexpressing the $O_2^{\bullet-}$ scavenger, superoxide dismutase (SOD), or receiving neocortical SOD application (Iadecola *et al*, 1999). Similarly, there is no impairment in the evoked CBF response to whisker stimulation or endothelium-dependent (ACh, bradykinin, and calcium ionophore A23187) and -independent (SNAP) vasoactive stimuli in young APP mice lacking the gp91^{phox} NADPH oxidase catalytic subunit (Park *et al*, 2005). Our own work in young APP mice (line J20; Mucke *et al*, 2000) corroborates the cerebrovascular dysfunction induced by soluble Aβ through oxidative stress. Dilatory responses to ACh and calcitonin gene-related peptide were diminished by ~50%, and there was reduced availability of NO, a vasodilator gas constitutively released by the endothelium and responsible, along with opposing effects from ET-1, for establishing vascular tone (Tong *et al*, 2005). Brief incubation of arterial segments with SOD or catalase that respectively eliminate $O_2^{\bullet-}$ and hydrogen peroxide radicals, quickly restored NO bioavailability and ACh-mediated dilatations. Similarly, the ACh response was recovered in cerebral arteries from old APP mice (over 18 months old) incubated with the NADPH oxidase inhibitor, apocynin (Hamel *et al*, 2008). The interaction between $O_2^{\bullet-}$ and NO yields peroxynitrite (ONOO-), detectable in the young APP vasculature as a nitrotyrosine immunoreactive product (Park *et al*, 2004; Tong *et al*, 2005). This oxidizing intermediate is capable of inflicting nitrosative stress on proteins like SOD (Guo *et al*, 2003) and on K_{ATP} channels that mediate calcitonin gene-related peptide relaxation in mouse cerebral arteries (Tong *et al*, 2009). Hence, eliminating $O_2^{\bullet-}$ can restore cerebrovascular function and prevent a self-perpetuating cycle of oxidative damage. Remarkably, and as a testament to the reversibility of this process, cerebrovascular rescue has been achieved even in aged APP mice (12 to 16 months old) after genetic (Park *et al*, 2008) or pharmacological interventions with antioxidants (Nicolakakis *et al*, 2008). The

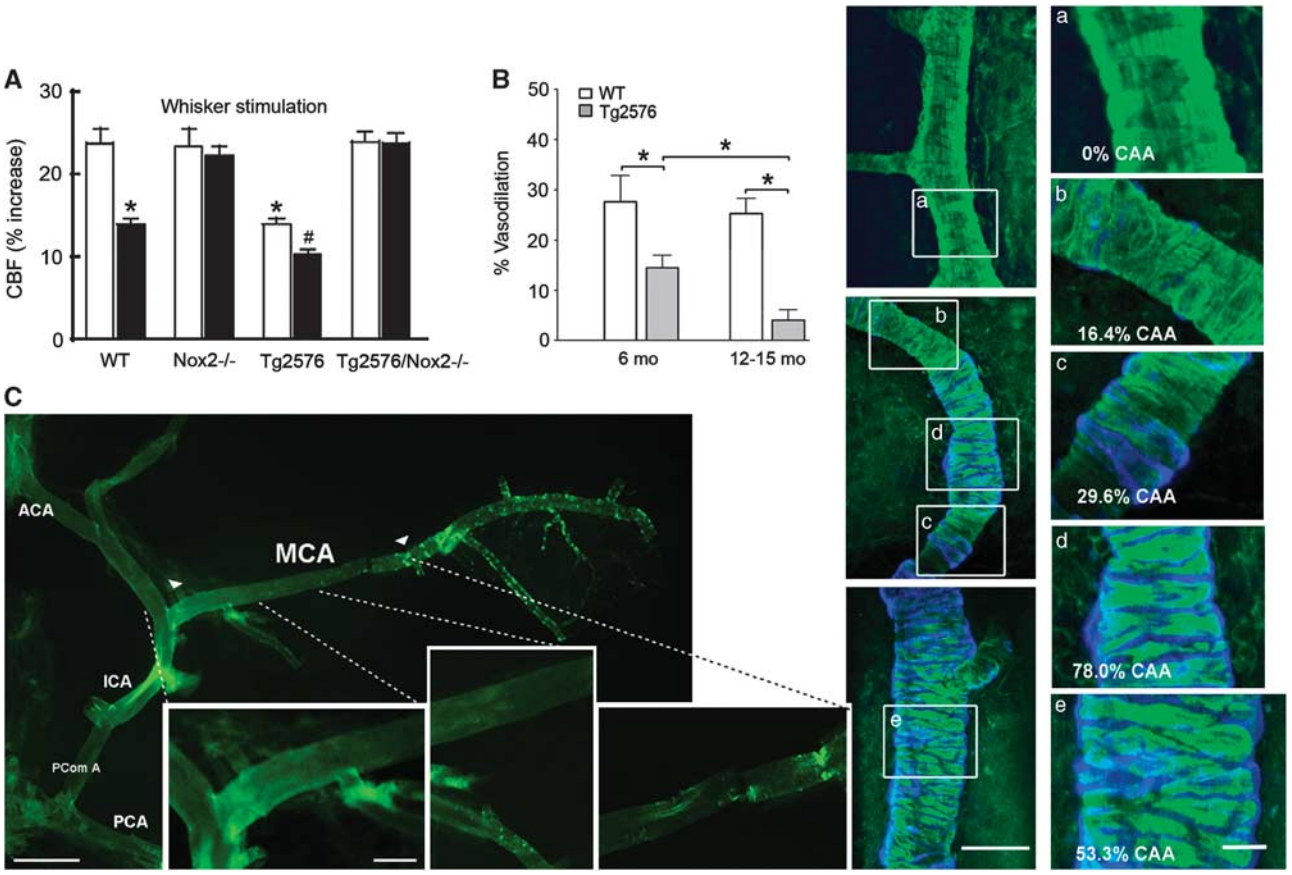

Figure 2 The role of soluble and insoluble Aβ in cerebrovascular dysfunction of amyloid precursor protein (APP) mice. (**A**) The cerebral blood flow (CBF) increase to whisker stimulation is impaired in 3- to 4-month-old Tg2576 mice lacking Aβ deposits, and more so in 12-month-old Tg2576 animals, as assessed through open cranial window and laser Doppler flowmetry (LDF). Deficits were absent in Tg2576 mice missing the Nox2 catalytic NADPH oxidase subunit. This implicates soluble Aβ-induced oxidative stress in the dysfunctions. White bars, 4 months old; Black bars, 12 months old; WT, wild type; *, different from WT; #, different from young Tg2576 mice. Reproduced from Park *et al* (2008) by The National Academy of Sciences of the USA. (**B**) Hypercapnia-induced dilatation is impaired in 6-month-old Tg2576 mice lacking cerebral amyloid angiopathy (CAA), and further impaired in 12- to 15-month-old animals with increasing CAA severity and smooth muscle cell disarrangement, the latter illustrated and quantified in panels (a–e). CAA: methoxy-X04 stained blue; smooth muscle cells: phalloidin stained green. Scale bars: left, 50 μm; right, 20 μm. Reproduced from Han *et al* (2008) with permission conveyed through Copyright Clearance Center, Inc. (**C**) Reduced cerebrovascular responsiveness in the proximal CAA-free segment of the middle cerebral artery (MCA) (delineated by arrowheads, with first, second, and third subsegments magnified in the three panels) suggests effects of soluble Aβ in a 12-month-old J20 APP mouse. Scale bars: left, 0.5 cm; right, 0.2 cm. ACA, anterior cerebral artery; ICA, internal carotid artery; PComA, posterior communicating artery; PCA, posterior cerebral artery. Reprinted from Tong *et al* (2009) with permission from Elsevier.

reversibility of cerebrovascular dysfunction at an advanced age when Aβ deposition, cholinergic denervation, and mnemonic impairment have developed is particularly relevant for AD patients who currently receive diagnosis at a late stage.

The above evidence suggests that cerebrovascular dysfunction in young APP mice lacking CAA is due to a free radical cascade triggered by soluble Aβ (Figure 2A). In older mice, the combined actions of soluble and deposited Aβ further impair vasodilatory and contractile capacity by damaging, and eventually destroying, vascular smooth muscle (Mueggler *et al*, 2003; Han *et al*, 2008; Park *et al*, 2008) (Figures 2A and 2B). In these mice, the effects of soluble Aβ can still be appreciated in CAA-free segments of the vasculature, when Aβ deposition is patchy and has not progressed to the entire arterial segment (Beckmann *et al*, 2003; Han *et al*, 2008; Tong *et al*, 2009) (Figure 2C). After this stage, both endothelium-dependent and -independent responses are impaired. Hence, Aβ-directed therapy to deplete soluble Aβ only partly restores function, suggesting irreversible CAA-induced damage (Han *et al*, 2008). However, the prevailing view of soluble Aβ vasoactivity has been challenged by studies showing the absence of cerebrovascular deficits after Aβ superfusion (Hu *et al*, 2008). Further, in 6- to 8-month-old Tg2576 mice, cerebrovascular responses to ACh and whisker stimulation were not different from those of WT littermates, and only became attenuated at 14 or 19 months of age (Christie *et al*, 2001; Shin *et al*, 2007). Authors credited the discrepancies to the

noninvasiveness of their procedures that avoided exposing the cortex, in contrast to previous open cranial window preparations. However, Han *et al* (2008) had used a closed cranial window technique to reveal impaired dilatations to ACh, SNAP, and hypercapnia in the same 6-month-old Tg2576 mice (Figure 2B). Work from our laboratory on the J20 APP model has shown impaired hyperemic responses to whisker stimulation at 12 and ~15 months of age using an intact skull preparation (Table 1) (Nicolakakis *et al*, 2008; Tong *et al*, 2009). APP mice carrying the vasculotropic London APP mutation further complicate the relationship between cerebrovascular function and Aβ. In these mice, resting CBF and hypercapnia measured by LDF were normal despite extensive CAA at 24 months of age (Van Dorpe *et al*, 2000). A comparative study of models and techniques might ultimately shed more light on these discrepancies.

Transforming growth factor-β1 mice: Investigations of the AD cerebrovascular pathology have been centered on CAA, especially in light of its undesirable increase during Aβ immunotherapy and the associated risk of cerebral hemorrhage in clinical trials (Boche *et al*, 2008). Amyloid precursor protein mice have been instrumental in such research. However, they do not reproduce the cerebrovascular fibrosis that also characterizes AD, and that some have hypothesized to be a factor in cerebrovascular Aβ deposition (Wyss-Coray *et al*, 1997). Transgenic mice overexpressing constitutively active TGF-β1 in astrocytes (TGF mice) were generated to clarify the role of TGF-β1 increase in AD, and more importantly, to address the cerebrovascular pathology related to the basement membrane (Wyss-Coray *et al*, 1995). The latter is thickened (Mancardi *et al*, 1980; Vinters *et al*, 1994) as a result of accumulation of collagen IV (Kalaria and Pax, 1995) and that of other matrix proteins, a phenomenon associated with high levels of TGF-β1 in AD vessels (Grammas and Ovase, 2002). Transforming growth factor mice feature increased expression of vascular growth factors (vascular endothelial growth factor; connective tissue growth factor), and accumulation of perlecan, fibronectin, laminin, and collagen in the vascular basement membrane that contribute to its thickening (Wyss-Coray *et al*, 1995, 2000; Tong *et al*, 2005; Nicolakakis *et al*, 2011). Not only do some of these proteins have the capacity to bind Aβ, and potentially initiate CAA (Castillo *et al*, 1997), but also their accumulation in capillary basement membranes could hinder substrate delivery and waste elimination across the blood–brain barrier. In line with this idea, TGF mice feature increased capillary basement membrane thickness and endothelial cell degeneration (Wyss-Coray *et al*, 2000), resting hypoperfusion throughout the brain (Gaertner *et al*, 2005), impaired neurovascular coupling during whisker stimulation (Nicolakakis *et al*, 2011) (Table 1), and reduced basal CGU (Galea *et al*, 2006). The hemodynamic and metabolic changes may be related to glial activation in the TGF mouse brain (Wyss-Coray *et al*, 1995; Lacombe *et al*, 2004; Nicolakakis *et al*, 2011), but are mainly believed to have a vascular etiology, in view of the unaltered neuronal indices in old transgenic animals relative to age-matched WT littermates (18 to 22 months old). At this age, TGF mice featured preserved cortical cholinergic innervation, intact CGU increase during whisker stimulation, as measured by ^{18}F-fluorodeoxyglucose-positron emission tomography, and preserved spatial memory in the Morris water maze (Nicolakakis *et al*, 2011). Mechanisms of TGF-β1-induced vascular dysfunction included reduced levels of endothelial nitric oxide synthase responsible for basal and stimulus-induced NO synthesis, decreased COX-2 protein levels, and deregulation of the contractile ET-1 signaling pathway (Tong *et al*, 2005; Tong and Hamel, 2007; Papadopoulos *et al*, 2010; Nicolakakis *et al*, 2011). In contrast to APP mice, *in vitro* and *in vivo* antioxidant treatments were unable to restore vascular reactivity in arteries from young and old TGF mice. Further, protein levels of the $O_2^{\bullet-}$ marker SOD2 were unchanged in TGF cerebral vessels (Tong *et al*, 2005; Nicolakakis *et al*, 2011). This collectively argued against oxidative stress as a deleterious factor in cerebrovascular function of TGF mice.

The lack of neuronal impairments in the TGF model demonstrated that neurovascular dysfunction could occur in the absence of neuronal compromise. This pointed to the potentially important role that TGF-β1-induced cerebrovascular alterations could play in AD neurovascular dysfunction. Further, TGF mice offered new insight into the hypoperfusion–dementia link. The lack of cholinergic, metabolic, and cognitive anomalies in aged TGF mice, despite chronic hypoperfusion that should negatively affect these parameters (Craft *et al*, 2005; Ruitenberg *et al*, 2005), suggested either insufficient CBF impairment or its role as an aggravating factor in an ongoing pathogenic process. The lack of mnemonic deficits may also have been attributable to a TGF-β1 neuroprotective role (Tesseur *et al*, 2006; Caraci *et al*, 2008; Cheng *et al*, 2009), although TGF-β1 neurodegenerative effects have also been reported (Salins *et al*, 2008; Town *et al*, 2008). Until this issue is clarified, TGF mice should be viewed as alternative models to classic artery occlusion paradigms that are invasive and often result in neurodegeneration and white-matter damage (Farkas *et al*, 2007; Barros *et al*, 2009; Miki *et al*, 2009), undesirable confounds in the hypoperfusion–dementia relationship. The TGF model should thus aid in the search for therapies against cerebrovascular dysfunction associated with TGF-β1 elevations and structural changes in AD.

Amyloid precursor protein/transforming growth factor-β1 mice: Transgenic mice simultaneously overproducing Aβ and TGF-β1 were created to investigate the relationship between cerebrovascular fibrosis and CAA (Wyss-Coray *et al*, 1997). This bitransgenic

APP/TGF model not only reflected the combined Aβ and TGF-β1 elevations encountered in AD patients, but also provided new mechanistic insight into AD neurovascular dysfunction. In young APP mice lacking cerebrovascular and parenchymal Aβ deposits, genetic addition of TGF-β1 induced CAA (Wyss-Coray *et al*, 1997). This suggested that cerebrovascular fibrosis could be a CAA trigger. Conversely, it also suggested that approaches targeting vascular wall thickening could reverse or prevent CAA, an idea that could be exploited in vaccination trials. In addition, vascular reactivity experiments from our group on isolated APP/TGF cerebral arteries revealed lower tonic NO levels and impaired vasodilatory capacity in response to ACh and calcitonin gene-related peptide starting at an early age. These deficits were associated with changes in vascular remodeling and signaling pathways, similar to those in TGF mice (Ongali *et al*, 2010). Further, the impaired ACh-mediated dilatations could not be rescued by incubation of arteries with apocynin or SOD, suggesting resistance to antioxidant approaches. This observation may help guide therapies for neurovascular dysfunction in AD. Progressive neurovascular coupling deficits to whisker stimulation in APP/TGF mice surpassed those of TGF mice at $\sim$18 months of age (Papadopoulos *et al*, 2010; Nicolakakis *et al*, 2011), and reached a severity parallel to that of similarly aged APP mice (Shin *et al*, 2007; Nicolakakis *et al*, 2008) (Table 1). The hemodynamic dysfunction was thought to arise from neuronal, astrocytic, and vascular impairments driven by increasing soluble Aβ levels, particularly those of $A\beta_{1-40}$ that continued to rise from 12 to 18 months of age, while those of $A\beta_{1-42}$ had stabilized (Ongali *et al*, 2010). Our ongoing work on APP/TGF mice will clarify whether *in vivo* pharmacological interventions can restore arterial reactivity and neurovascular coupling, as well as improve the impaired cholinergic innervation, neurometabolic coupling, and spatial memory of the mice.

We have provided an overview of neurovascular function in transgenic models for which data were available. However, a similar evaluation would be particularly interesting in LaFerla's triple transgenic model (Oddo *et al*, 2003), which harbors human mutant APP, presenilin 1, and τ transgenes, and develops the neurofibrillary tangle pathology not seen in APP mice. The model could yield valuable insight on the potential effect of cytoskeletal and axonal transport deficits on neurovascular coupling. It could also elucidate the impact of hyperphosphorylated τ and neurofibrillary tangles on basal forebrain cholinergic neurons, which modulate neurovascular coupling. Significant axonal transport deficits and τ hyperphosphorylation were reported by Massaad *et al* (2010) in 8 and 12- to 16-month-old Tg2576 mice. Authors determined axonal transport rates by visualizing the rate of Mn^{2+} accumulation in olfactory bulb with magnetic resonance imaging, after application of a manganese chloride ($MnCl_2$) solution to mouse nostrils.

Treatments for Alzheimer's disease neurovascular dysfunction

In APP mice, antioxidant approaches have proven beneficial against neurovascular dysfunction. Superfusion of the free radical scavenger MnTBAP on the somatosensory cortex restored neurovascular coupling during whisker stimulation in aged (12 to 15 months old) Tg2576 animals (Park *et al*, 2008). Similarly, we observed recovery of the functional hyperemic response in $\sim$8-month-old J20 APP mice after brief *in vivo* treatment with the SOD mimetic

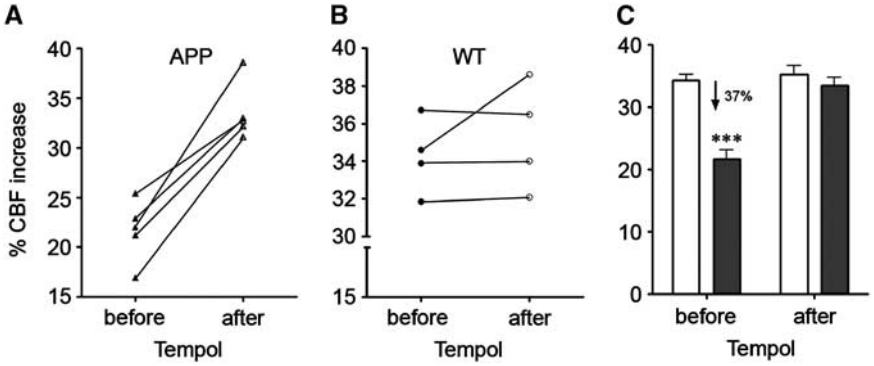

Figure 3 Functional hyperemic rescue by Tempol in 7.5-month-old J20 amyloid precursor protein (APP) mice. (**A**) The neurovascular coupling response to whisker stimulation was improved in all APP mice tested before and after 10-day *in vivo* therapy with the antioxidant Tempol (1 mmol/L in drinking water, Sigma-Aldrich, St Louis, MO, USA), as measured with laser Doppler flowmetry (LDF), following the same protocols as in Nicolakakis *et al* (2008) ($n = 5$, paired Student's *t*-test, GraphPad Prism 4, San Diego, CA, USA). (**B**) Responses of wild-type (WT) mice were mostly unaffected by treatment ($n = 4$, paired Student's *t*-test). (**C**) Short Tempol therapy completely normalized the impaired CBF response of APP mice (gray bars, $n = 5$) to that of WT littermates (white bars, $n = 4$) (***$P < 0.001$ for difference to all other groups, two-way analysis of variance with Newman–Keuls *post hoc* multiple comparison test (Statistica 9, StatSoft, Tulsa, OK, USA)). Error bars represent s.e.m. Results are expressed as the percent cerebral blood flow (CBF) increase during whisker stimulation relative to prestimulation baseline.

Tempol. In this experiment, the evoked CBF response to whisker stimulation was measured with LDF in the same animals before and after 10-day Tempol therapy. We found that the antioxidant significantly improved neurovascular coupling in every APP mouse tested ($t=7.311$, df$=4$, $P<0.01$), such that it completely normalized the impaired CBF response of APP mice (-37%, $P<0.001$; Table 1, Figure 3) to the level of WT littermates (Figure 3). There was no drug effect on the WT response ($t=1.041$, df$=3$, $P=0.374$). Such antioxidant approaches have not, to our knowledge, been verified in humans for their effect on evoked perfusion. But given their overall inefficacy in AD trials (Petersen *et al*, 2005), and on cerebrovascular function of TGF and APP/TGF mice, alternative therapies should be pursued. In the section that follows, we review two drug classes with reported benefits on brain hemodynamics and neurovascular coupling in AD patients. Their mechanisms of action have been elucidated to varying extent in the mouse models.

Cholinesterase Inhibitors

Cholinesterase inhibitors are the standard FDA-approved symptomatic therapy for mild-to-moderate cases of AD. Based on the premise that reduced cholinergic tone is partly responsible for attention deficits and cognitive decline in AD, these compounds were developed to prolong the synaptic life of ACh. Four are available, tacrine (Cognex, Parke-Davis Pharmaceuticals), donepezil (Aricept, Pfizer Inc.), rivastigmine (Exelon, Novartis Pharmaceuticals Corporation), and galantamine (Reminyl, Janssen Inc.), although the first of these is no longer used after liver-toxicity side effects. A Cochrane review found that the three compounds display a small but short-lived cognitive benefit (Birks, 2006). In light of renewed interest in the AD circulation, cholinesterase inhibitors were revisited and found to preserve or improve perfusion in AD patients undergoing therapy (Nakano *et al*, 2001; Nobili *et al*, 2002; Rosengarten *et al*, 2006; Bär *et al*, 2007; Yoshida *et al*, 2007) (Figure 4). In the study by Bär *et al* (2007), 5-week galantamine administration improved CO_2 reactivity as measured in the middle cerebral artery of 17 AD patients with transcranial Doppler sonography. In contrast, galantamine had no effect on cerebral autoregulation and cortical hemoglobin decrease elicited in 13 AD patients during orthostatic hypotension. Hence, it may not offer protection against increased ischemic vulnerability during common activities such as standing up (Van Beek *et al*, 2010). Rosengarten *et al* (2006) found that 2-month donepezil therapy normalized the evoked flow response in the posterior cerebral artery during a reading task. Together, these studies suggested that some of the cognitive benefits of cholinesterase inhibition may arise from direct effects on the vasculature, as had been proposed by Claassen and Jansen (2006) in their cholinergic-vascular hypothesis. This idea was further supported by the finding that patients whose cognitive score had stabilized or improved with cholinesterase inhibitor therapy, the so-called 'responders,' were also those who featured stabilized or increased perfusion, often within the first month of a 1-year treatment. Patients whose CBF had decreased during therapy were also those who had declined cognitively, the designated 'nonresponders' (Nakano *et al*, 2001; Nobili *et al*, 2002; Yoshida *et al*, 2007). The latter data highlighted the importance of targeting perfusion deficits as a means to influence mental status. They also emphasized the value of CBF as a correlate and possible predictor of treatment outcome. However, the limited benefits currently afforded by cholinesterase inhibition also point to the need for more effective therapies and for earlier detection.

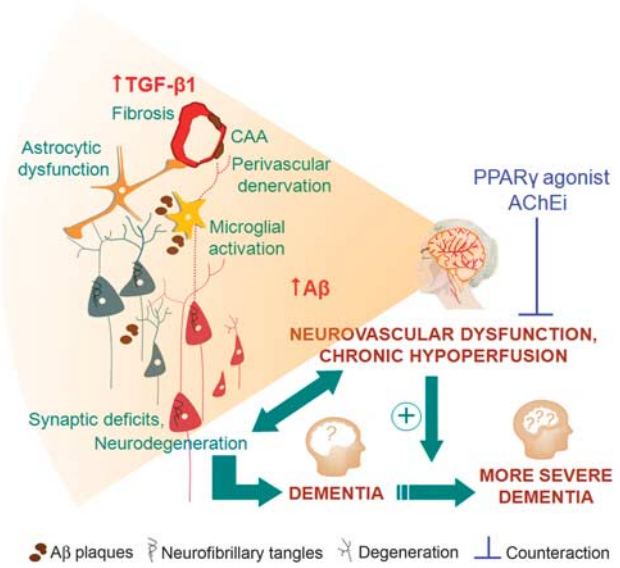

Figure 4 Alzheimer's disease (AD) neurovascular dysfunction and chronic hypoperfusion are associated with diseased neuronal, astrocytic, and vascular networks. Countering neurovascular impairment with peroxisome proliferator-activated receptor γ (PPARγ) agonists, acetylcholinesterase inhibitors (AChEi), or other compounds may improve clinical outcome and delay progression to severe dementia. CAA, cerebral amyloid angiopathy; TGF-β1, transforming growth factor-β 1.

Peroxisome Proliferator-Activated Receptor γ Agonists

Agonists of the peroxisome proliferator-activated receptor γ form a class of oral antidiabetic drugs that were introduced in the late 1990s for the treatment of type 2 diabetes. Rosiglitazone (Avandia, GlaxoSmithKline) and pioglitazone (Actos, Takeda Pharmaceuticals, North America, Inc.) are the two prescribed thiazolidinedione peroxisome proliferator-activated receptor γ agonists that act by enhancing insulin sensitivity. However, their benefits in AD are believed to derive from their ability as nuclear receptor ligands to regulate transcription of

a wide variety of oxidative, inflammatory, fibrotic, and neuronal survival genes, although transcription-independent effects may also be involved. Peroxisome proliferator-activated receptor γ agonists thus have the ability to improve neuronal, glial, and cerebrovascular networks in AD, and consequently to rescue brain hemodynamics (reviewed in Nicolakakis and Hamel (2010)) (Figure 4). Indeed, pioglitazone was recently shown to ameliorate cerebral perfusion in AD patients (Sato *et al*, 2009) and in transgenic mouse models (Nicolakakis *et al*, 2008, 2011). In Sato *et al* (2009), diabetic AD patients treated with pioglitazone for 6 months experienced regional CBF increase in the parietal lobe, concomitant with an improvement in cognition, whereas no such effects were seen in placebo-treated subjects. At the same time, fasting plasma insulin levels declined in pioglitazone-treated patients, indicating enhanced insulin sensitivity that could have led to some of the observed cognitive benefits. These findings are supported by data in aged APP mice treated with pioglitazone; cerebrovascular reactivity and evoked CBF and CGU responses were normalized, the latter respectively determined by LDF and ^{18}F-fluorodeoxyglucose-positron emission tomography (Nicolakakis *et al*, 2008). However, despite these favorable effects on the cerebral circulation, and attenuation of oxidative stress and neuroinflammation, pioglitazone failed to rescue memory deficits. We originally attributed this failure to the short treatment duration (6 weeks), advanced aged of the mice (>15 months), and highly stringent conditions of the Morris water maze. Our more recent study in young APP mice treated for 3 months with a higher pioglitazone dose similarly failed to show notable cognitive improvement (Badhwar *et al*, 2010), suggesting that pioglitazone is unlikely to be a robust therapeutic approach to AD. Similarly, the latest pioglitazone pilot study in nondiabetic AD patients found no cognitive benefit (Geldmacher *et al*, 2011). In aged TGF mice, pioglitazone countered diminished arterial reactivity, glial activation, and neurovascular coupling deficits, despite persisting cerebrovascular fibrosis (Nicolakakis *et al*, 2011). This revealed additional mechanisms by which thiazolidinediones may restore cerebrovascular function in AD patients featuring TGF-β1 elevations, vascular wall thickening, and vascular antioxidant resistance. Despite cerebrovascular improvements, the collective literature casts doubt on the ultimate benefit of thiazolidinediones in AD. The magnitude of improvement reported in previous clinical trials with rosiglitazone (Watson *et al*, 2005; Risner *et al*, 2006; Gold *et al*, 2010) and pioglitazone (Hanyu *et al*, 2009; Sato *et al*, 2009) was not superior to that offered by cholinesterase inhibitors. Coupled to rosiglitazone cardiovascular side effects and potential removal from the North American market, we remain skeptical about the clinical value of thiazolidinediones. However, the safer cardiovascular profile of pioglitazone (Lincoff *et al*, 2007) may make it advantageous for subpopulations, for example diabetic AD patients, who could avoid cholinesterase inhibitor side effects in favor of drugs that also offer glycemic control.

Conclusion

Decreases in resting CBF and alterations in evoked responses, in particular the inability to silence task-irrelevant networks, are preclinical hemodynamic features that may be used to predict pathological conversion to AD from a presymptomatic state. They also correlate with therapeutic outcome, as shown with regards to cholinesterase inhibitors and peroxisome proliferator-activated receptor γ agonists. Such observations have prompted renewed interest in the AD circulation and the continued use of experimental models to explore and counter pathological changes in neuronal, astrocytic, and vascular compartments. Superfusion or overexpression of key vasoactive AD proteins has provided mechanistic insight and experimental evidence that therapeutic intervention is feasible, yet a disease-modifying treatment remains elusive, and earlier intervention may ultimately have greater impact in patients. Devising strategies to protect blood supply to an organ with very high energetic demands and lack of reserves should counter AD neurovascular dysfunction, and help delay disease progression and cognitive demise. Improved therapeutic end points may be better achieved by combined regimens, early intervention, and selection of subgroups of AD patients.

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