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Neurovascular Mechanisms in Stroke, Neurodegeneration and Recovery

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The emerging concept of the "neurovascular unit" may enable a powerful paradigm shift for neuroscience. Instead of a pure focus on the "neurobiology" of disease, an opportunity now exists to return to a more integrative approach. The neurovascular unit emphasizes that signaling between vascular and neuronal compartments comprise the basis for both function and dysfunction in brain. Hence, brain disorders are not just due to death of neurons, but instead manifested as cell signaling perturbations at the neurovascular interface. In this mini-review, we will examine 3 examples of this hypothesis: neurovascular mechanisms involved in the thrombolytic therapy of stroke, the crosstalk between neurogenesis and angiogenesis, and the link between vascular dysfunction and amyloid pathology in Alzheimer's disease. An understanding of cell-cell and cell-matrix signaling at the neurovascular interface may yield new approaches for targeting CNS disorders.

Key Words: Neurovascular unit, Tissue plasminogen activator, Stroke, Matrix metalloproteinase, Alzheimer's disease, Neurogenesis

INTRODUCTION

Over the past decade, major advances in the neurobiology of CNS disorders were achieved. Fundamental mechanisms underlying neuronal cell death were elucidated, including excitotoxicity, oxidative damage via free radicals, and apoptotic-like events that are triggered after brain injury. Altogether, these advances have revealed a plethora of therapeutic targets for the brain. For example, it was demonstrated that after onset of cerebral ischemia in experimental animal models, interstitial glutamate concentrations rise dramatically to neurotoxic levels (Buchan, 1990; Benveniste, 1991; Lipton & Rosenberg, 1994). Blockade of glutamate receptors of the NMDA or AMPA subtypes all seem to reduce brain infarction in animal stroke models (Hirose & Chan, 1993). Disappointingly, however, a wide range of clinical stroke trials testing glutamate receptor antagonists have failed to show efficacy in patients.

Why have these therapeutic approaches not achieved significant success? Whereas in isolated cell cultures, specific neuronal death pathways were clearly validated, the pharmacologic probes developed based on these paradigms have not translated well into the clinic. There are many reasons why clinical trials of neuroprotection are inherently challenging, and the reader is referred to many other more detailed reviews on this topic (De Keyser et al, 2000; Gladstone et al, 2002). However, an emerging concept that has gained momentum in recent years is the realization that the brain is not a neuron! Brain function and dysfunction arises from a complex interplay between a network of multiple cell types, including neurons, astrocytes, oligodendrocytes, microglia, and ultimately, the cerebrovasculature that permeates the entire organ.

From a functional perspective, it is the interaction between neuron and astrocyte that mediates neurotransmitter release and reuptake at the synapse. The integrity of the blood-brain barrier depends on cell-cell signaling between the astrocyte and the cerebral endothelium at the microvessel level. And the impressive advances in functional MRI reveal the intricacies of brain function would not be possible without the hemodynamic coupling between neuronal firing and vascular response. A simplified schematic in Fig. 1 summarizes these interactions.

The neurovascular unit provides a conceptual framework that emphasizes cell-cell signaling in the brain (Lo et al, 2004; Hawkins & Davis, 2005; Allan, 2006). Brain disease is therefore manifested as a pdlferturbation in signaling within the cells of the neurovascular unit (Iadecola, 2004; Zlokovic, 2005; Abbott et al, 2006). Even though we have made enormous advances in understanding intra-neuronal mechanisms of cell death, we may have to enlarge our focus to the level of the neurovascular unit if we are to make a difference at the organ level. In this mini-review, we will briefly examine 3 "case studies" of the neurovascular unit: (a) the pleiotropic actions of various proteases during thro-

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ABBREVIATIONS: NMDA, N-methyl-D-asparate; AMPA, alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate; CNS, central nervous system; LRP, low density lipoprotein receptor related protein; BBB, blood brain barrier; MMP, matrix metalloproteinase; tPA, tissue plasminogen activator; MRI, magnetic resonance imaging; PET, positron emission tomography.

mbolysis that may explain responses in stroke therapy, (b) the interplay between neurogenesis and angiogenesis in brain remodeling after injury, and (c) the cerebrovascular response to amyloid in Alzheimer's disease.

Tissue Plasminogen Activator: A Pleiotropic Neurovascular Mediator in Stroke

Historically, two phenomenon from animal models suggested that active cell death mechanisms are triggered after cerebral ischemia. In the late 1970's, Astrup, Symon and colleagues demonstrated that the ischemic penumbra comprised transiently viable tissue whereby moderate ischemia resulted in loss of evoked potentials but not resting membrane potentials (Astrup et al, 1977). With a return of blood flow, penumbral evoked potentials could be rescued. But without reperfusion, the penumbra would collapse over time and anoxic depolarization takes place. The second phenomenon involved selective neuronal vulnerability, whereby pyramidal neurons in the CA1 sector of the hippocampus underwent delayed cell death within 2~3 days after a transient global cerebral ischemic insult (Garcia, 1988; Paschen, 1996; Harukuni & Bhardwaj, 2006). Taken together, these observations supported the hypothesis that complex and undefined mechanisms are indeed activated that eventually leads to cell death after stroke.

Whatever these neuronal mechanisms might be, a logical therapeutic approach to cerebral ischemia is to restore blood flow. Therefore, thrombolysis with tissue plasminogen activator (tPA) is a rational therapy, and in properly selected patients, tPA works very well (ECASS Study Group, 1995; NINDS rt-PA Stroke Study Group, 1995). However, many limitations still exist. The treatment time window is exceedingly narrow. Not all patients respond. And there remains an overall risk of edema and hemorrhagic conversion (Hacke et al, 1999). Over the past several years, emerging data from cell and animal model systems now suggest that these caveats with tPA stroke therapy might be due to the fact that tPA is not only a "blood molecule" but also has neuroactive properties (Kaur et al, 2004; Benchenane et al, 2005). In this regard, tPA is perhaps best understood as a neurovascular mediator with pleiotropic actions precisely at the critical neurovascular interface.

The primary goal of using tPA in stroke is straight-forward. By converting plasminogen into active plasmin, fibrin is degraded and the offending embolic clot is dissolved. But tPA may do much more. tPA has been shown to be vasoactive. Depending on the concentrations, tPA can be either vasodilatory or vasoconstrictive (Nassar et al, 2004). tPA may also be a critical neuronal mediator that is released in a calcium dependent manner (Gualadris et al, 1996). The plasminogen protease system may play a key role in modulating extracellular microenvironment during synaptic remodeling. Indeed, tPA knockout mice show perturbations in several paradigms of long term potentiation (Baranes et al, 1998).

Tsirka and colleagues demonstrated that tPA can be deleterious to neurons. After kainic acid injections into the hippocampus, tPA deficient knockout mice were resistant to excitotoxic injury compared with wildtype mice (Tsirka et al, 1995; Tsirka et al, 1996). Subsequently, Tsirka, Strickland and Lipton showed that these neuronal effects of tPA were also implicated in stroke; tPA knockout mice suffered significantly reduced infarctions after focal cerebral ischemia (Wang et al, 1998). The underlying mechanisms may involve anoikis since degradation of inter-neuronal laminin seemed to be involved (Chen & Strickland, 1997). Additionally, a linkage between proteolysis and excitotoxicity may also contribute. Vivien and colleagues showed that tPA interacted with the NR1 subunit of the NMDA receptor complex (Nicole et al, 2001). Cleavage of this subunit amplifies calcium currents which may augment excitotoxicity. This novel mechanism may serve to explain why tPA may be neurotoxic under certain conditions. More recently, Tsirka and colleagues have also demonstrated that tPA may behave as a chemokine since sources in damaged brain can activate microglia and trigger downstream neuroinflammation (Rogove et al, 1999; Wang et al, 2003a).

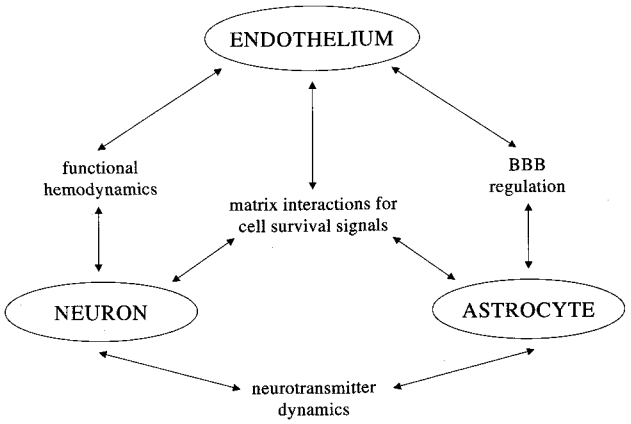

Fig. 1. Schematic of the neurovascular unit, emphasizing the functional aspects of cell-cell and cell-matrix signaling. Note that white matter is not included in this initial simplified schema. A full dissection of signaling between multiple glial cell types should also be considered. Adapted from Lo et al, *Stroke* 2005.

In addition to neuronal processes, tPA can also trigger changes in other protease systems. A major hypothesis is that complications of bleeding and edema after thrombolysis is due to signaling connections between the plasminogen system and the matrix metalloprotease (MMP) system. MMP inhibitors reduce tPA-associated hemorrhage in embolic stroke models (Lapchak et al, 2000; Sumii & Lo, 2002). It was demonstrated that tPA can bind to the LRP lipoprotein receptor and induce a transcriptional upregulation in MMPs (Wang et al, 2003b). tPA knockout mice have reduced MMPs and brain edema after cerebral ischemia (Tsuji et al, 2005). These experimental notions are now supported by accumulating clinical data. Stroke patients with increased MMPs are more likely to have worse outcomes, including increased risk of hemorrhagic conversion (Montaner et al, 2001; Montaner et al, 2003; Alvarez-Sabin et al, 2004). Acute stroke patients that receive tPA seem to have elevated MMP-9 levels (Ning et al, 2006). More recently, elevated plasma MMP-9 levels have been correlated with MMP-9 staining in human stroke brain samples as well (Rosell et al, 2006; Tejima et al, 2006). Taken together, these data raise the possibility that MMP inhibition should be considered as part of a combination stroke therapy together with tPA thrombolysis.

Recent neuroimaging data may further support this idea. Warach, Latour and Kidwell et al showed that leakage of gadolinium contrast can be detected with FLAIR MRI during the hyperacute to acute progression of ischemic stroke

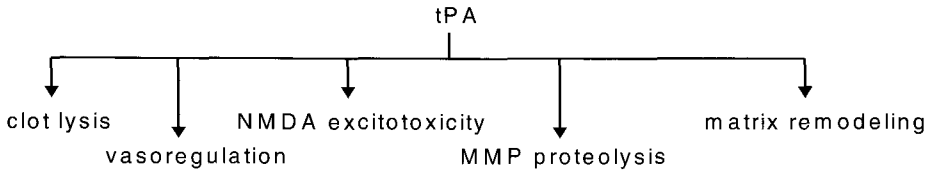

Fig. 2. Multiple actions of tPA at the neurovascular interface. In addition to its intended role as a clot-lysing agent, tPA can also be vasoactive, neuromodulatory, and by activating the MMP protease system, also mediate acute BBB damage or chronic neurovascular remodeling. Adapted from Wang et al, *Stroke* 2004.

(Warach & Latour, 2004). They have termed these subtle enhancement signals HARM or Hyperacute Reperfusion Marker. HARM seems to be correlated with negative outcomes including hemorrhage and edema observed with increased T2 at later times. Interestingly, when tPA reperfusion was compared with reperfusion using mechanical clot remover devices, HARM signals were increased. These initial data suggest that reperfusion with tPA is biologically different from reperfusion via mechanical clot removal.

Altogether, these data indicate that tPA may have multiple actions in brain (Fig. 2). By understanding these complex neurovascular interactions that are affected by tPA, we may be able to better modulate its actions, and hopefully design combination stroke therapies that target the entire neurovascular unit in addition to lysing the clot.

The Neurovascular Niche for Neurogenesis and Brain Remodeling

For many decades, a dogma in neurobiology stated that no new neurons are born in adult mammalian brain. However, beginning in the 1960's and early 1970's, challenges to this dogma began to surface, with initial data coming from the radiobiology literature (Lewis, 1968; Hopewell & Cavanagh, 1972; Privat & Leblond, 1972). DNA labeling studies indicated that in fact, there was significant cell turnover in selected sites of mouse and rat brain, specifically in the hippocampus and subventricular areas. The initial thrust of these early studies focused on the basic idea that radiation injury primarily induced a "reproductive death", i.e. DNA damage was typically not severe enough to induce outright cytotoxicity, and DNA misrepair problems only surfaced when each cell attempted to divide. The surprising radiosensitivity of adult brain suggested that cell division remained intact.

The wider implications of these observations were not fully appreciated until the late 1980's and early 1990's. Snyder, Cepko and colleagues discovered that neuroblast-like precursor cells could be isolated from mammalian brain (Ryder et al, 1990). The multipotent nature of these precursor cells were demonstrated by Weiss and colleagues; isolated cultures could be differentially induced to mature into either new neurons or astrocytes (Reynolds & Weiss, 1992). Taken together, these data led to the idea that pockets of ongoing neurogenesis (and gliogenesis) may persist in adult brain.

The concept of a vascular niche for neurogenesis was first proposed by Palmer, Gage and Goldman. It was discovered that endothelial production of brain-derived neurotrophic factor significantly enhanced neural precursor turn-over in cell cultures (Leventhal et al, 1999). In vivo, detailed microscopic analysis revealed that there were close spatial relationships between sites of neurogenesis and active microvasculature, suggesting a functional interdependence between the two mechanisms (Palmer et al, 2000). Mechanistically, it was ultimately demonstrated that conditioned media from cerebral endothelial cells directly induced neuroblast proliferation (Shen et al, 2004). From an evolutionary perspective, similarities between the morphologic development of the nervous system have long been recognized to parallel the intricate branching profiles of the vascular network (Carmeliet, 2003; Eichmann et al, 2005). Increasingly, a molecular dissection of the signals reveal mechanistic overlaps between angiogenic and neurogenic mediators, further supporting the fundamental concept that one cannot examine neurogenesis and angiogenesis separately, but instead one has to consider neurovascular development as a coordinated phenomenon in itself. In adult brain, these trophic and signaling connections may also be vital for homeostasis, as proposed by LaManna et al and Kim and colleagues (Park et al, 2003; Ward & Lamanna, 2004) (Fig. 3).

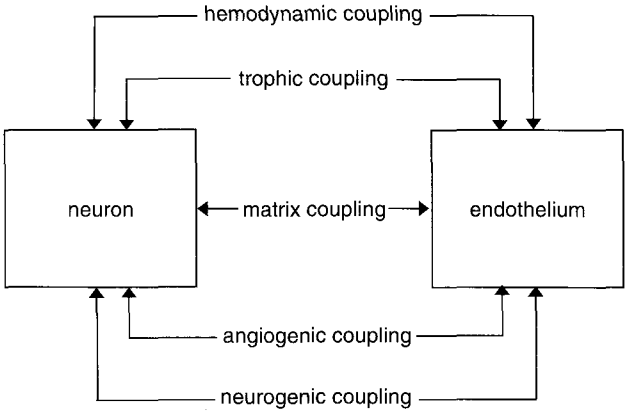

Fig. 3. Schematic emphasizing that neurovascular coupling can be manifested at multiple levels. Traditional hemodynamic couping links neuronal activity with blood flow responses that occur at millisecond timescales. Trophic-matrix coupling at longer distances and timescales as diffusible mediators may provide signals to coordinate neurogenesis and angiogenesis. Based on fundamental concepts first outlined in Park et al, *Biochem Biophys Res Commun* 2003, and Ward and LaManna, *Neurol Res* 2004.

From a clinical standpoint, interactions between neurogenesis and angiogenesis not only contribute to brain development, but also influence what happens during recovery from brain injury. As discussed earlier, disruptions in neurovascular proteases such as MMPs mediate acute damage. However, because these neurovascular proteases mediate

angiogenesis, vasculogenesis and neurogenesis in developing brain, it is likely that they may also play key roles during plasticity and remodeling.

Several labs simultaneously showed that neurogenesis was upregulated after cerebral ischemia. Liu, Sharp and colleagues reported that rates of neuroblast turnover were increased in the hippocampus after transient global cerebral ischemia (Liu et al, 1998). Chopp and colleagues showed that subventricular zone cell kinetics responded sharply after focal ischemia (Zhang et al, 2001). And subsequently, the Lindwall group and the Parent lab both obtained powerful evidence in rat stroke models that increased neuroblast migration was diverted away from the baseline rostral migratory stream toward damaged striatum (Arvidsson et al, 2002; Parent et al, 2002). How do these neuroblasts move? Is it possible that MMPs may also be involved? A recent study showed that doublecortin-positive neuroblasts co-localize with MMP-9 staining (Lee et al, 2006). And broad spectrum inhibition of MMPs significantly thwarted the migratory response of these neuroblasts. Ultimately, the ability of using growth factor supplementation to amplify these neurogenic and presumably angiogenic responses provides promise that therapies for acute stroke and trauma might eventually move into the chronic phase, where one might even speculate about regrowing brain.

Besides the remote migration of newborn cells in damaged Brain, it is also recognized that important neurovascular responses may occur in peri-infarct cortex. Stroke recovery may be based in part on these morphologic substrates, as detected with functional MRI or PET (Dijkhuizen et al, 2003; Kim et al, 2005; Kim et al, 2006). Once again, a biphasic role for neurovascular proteolysis may occur. Zhao et al showed that many weeks after stroke, the peri-infarct cortex remains a dynamic and highly malleable territory (Zhao et al, 2006). Secondary elevations in MMPs are readily apparent, and these signals co-localize with surrogate markers of dendritic and microvessel regrowth. Consequently, inhibition of these MMPs during the delayed phase after stroke made things worse. Markers of neurovascular remodeling were suppressed, and infarcts and cavitations became larger. Furthermore, a significant number of animals showed signs of the development of abnormal and hemorrhagic blood vessels.

The intimate connections between neurogenesis and angiogenesis are critical not only during brain development, but also play a role in remodeling as the brain tries to heal itself after injury, neurodegenerative disease and perhaps even aging. In part, initial data suggest that neurovascular proteases such as MMPs may be involved. Thus, MMP inhibition might reduce hemorrhage during acute brain injury, a delicate promotion of endogenous MMP activities may be required for functional recovery and the matrix integration between neurogenesis and angiogenesis.

Vascular Correlates of Amyloid Neuropathology

Alzheimer's disease is a major cause of dementia in aging populations. Currently, the major pathogenic theories of Alzheimer's are focused on amyloid accumulation and altered tau processing in neurons. A full review of these molecular mechanisms that underlie Alzheimer's is outside the scope of this mini-review, and the reader is referred to many excellent reviews on this subject (Selkoe, 1999; Bossy-Wetzel et al, 2004; Mattson, 2004). However, an emerging set of ideas over the past 5~6 years now propose that in addition to a pure neuronal disease, Alzheimer's may also have key vascular correlates that must be considered if we are to find the most efficacious treatments for this devastating disorder (Iadecola, 2004; Zlokovic, 2005; Park et al, 2006).

From a purely epidemiological perspective, it is useful to note that major risk factors for sporadic Alzheimer's are mostly cardiovascular in nature (de la Torre, 2002). The prominent Rotterdam study, Honolulu study and others have documented that increased Alzheimer's risk was correlated with hypertension, atrial fibrillation, elevated homocysteine, diabetes, smoking, thrombosis, and atherosclerosis (Breteler, 2000). Increasingly, the research community is acknowledging that there are blurred boundaries and many overlaps between "true" Alzheimer's dementia and vascular-related dementia. PET imaging studies have long demonstrated that early perturbations in cerebral blood flow and metabolism, especially in frontal and temperoparietal cortex, were hallmarks of Alzheimer' s and neurodegeneration (de la Torre, 2002). And ultimately, the cognitive deficit profiles in vascular dementia, Alzheimer's and mild cognitive impairment all suggest a spectrum of dysfunction rather than strict and clear-cut categorical differences per se.

A classic example might perhaps be found in analysis of transgenic mice that overexpress amyloid precursor protein (McGowan et al, 2006). As expected, plaques accumulate at later stages of life, corresponding with behavioral defects and neuronal dysfunction. However, a closer look at these brains suggest that vascular changes may occur even earlier. Markers of oxidative and nitrosative stress, as indicated by nitrotyrosine formation, became apparent even before extracellular amyloid deposits occurred (Park et al, 2004). Furthermore, it was shown that critical problems with neurovascular coupling were present in these mice. Facial whisker stimulation is an established paradigm for activating whisker barrel cortex in rodent brains. Realtime measurements of cerebral blood flow using laser Doppler techniques demonstrated that the hemodynamic response coupled with neuronal activation seemed to be significantly suppressed in Alzheimer transgenic mice (Niwa et al, 2002a; Niwa et al, 2002b). The involvement of free radicals was validated when functional rescue of vascular deficits could be achieved by upregulating superoxide dismutase (Iadecola et al, 1999). Taken together, these data strongly suggest that problems in neurovascular coupling may play a central role in the pathology of progressive Alzheimer's disease.

The importance of the cerebrovasculature can also be interpreted in terms of amyloid clearance and kinetics between blood and brain. A rigorous review of these vital concept may be found in a review by Zlokovic (Zlokovic, 2005). Briefly, the amyloid content of brain is not static. Instead there is a dyamic equilibrium and exchange between parenchymal and vascular compartments. This process can be demonstrated by the passive immunization strategy, whereby blood-borne depletion of amyloid may help create gradients so that brain amyloid can be cleared as well. In aging and diseased brain, the receptors responsible for mediating amyloid transport may be altered; these include lipoprotein receptors and RAGE (Davis et al, 2004; Deane et al, 2004). Ultimately, increased deposition in concert with decreased clearance may accelerate the progression of disease and neurodegeneration (Silverberg et al, 2003; Donahue et al, 2006).

In terms of amyloid exposure per se, it may be useful

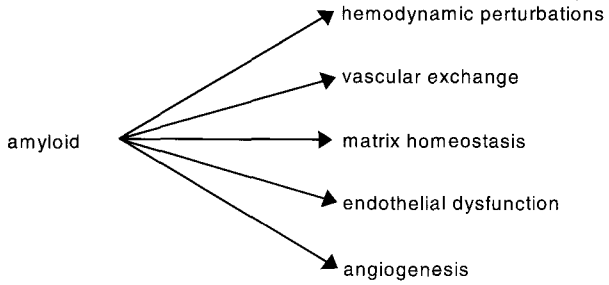

Fig. 4. Hypothesized vascular effects of amyloid. Besides a neurocentric focus, these interactions at the level of the entire neurovascular unit must also be addressed in the search for Alzheimer therapies. Based in part on fundamental concepts outlined by Zlokovic, Trends Neurosci 2005, and Iadecola, Nat Rev Neurosci 2004.

to note that vascular deposits may also be critical. The close association between Alzheimer's dementia and cerebral amyloid angiopathy may yet provide another model system for testing our neurovascular hypotheses (Greenberg, 2002; Zhang-Nunes et al, 2006). Amyloid can disrupt regulated blood flow (Niwa et al, 2002a; Niwa et al, 2002b), and higher levels can trigger apoptotic pathways in cerebral endothelial cells (Xu et al, 2001; Yin et al, 2002; Yin et al, 2005). From a therapeutic perspective, is it possible to hope that targeting endothelium might be more accessible than trying to salvage neurons lying behind the blood-brain barrier?

Once the degenerative process has been initiated, the brain should respond in an effort to restore homeostasis. Therefore, another hypothesis worth pursuing might involve reactions in brain cell turn-over. Baseline neurogenesis is perturbed in Alzheimer brains, although the pro-neurogenic versus anti-neurogenic actions of amyloid remain to be fully clarified (Greenberg & Jin, 2006). More recently, it has also been proposed that amyloid may have effects on angiogenesis (Vagnucci & Li, 2003; Zlokovic, 2005), so these responses in Alzheimer brains may have to be re-interpreted in the context of an integrated milieu of neurovascular remodeling.

It may be interesting to link these notions of neurovascular remodeling to the fact that ApoE isoforms constitute risk factors of Alzheimer's disease (Raber et al, 2004). Although the precise correlations are complex and it is difficult to establish causality, one might simplify the situation to say that ApoE4 isoforms increase the risk of "true" Alzheimer's disease, whereas ApoE2 isoforms may increase the risk of angiopathy and hemorrhage. Recently, it has been proposed that reactive astrocytes may help degrade amyloid, perhaps via MMPs (Deb et al, 2003; Wyss-Coray et al, 2003; Yan et al, 2006). Cell culture studies demonstrate that amyloid induces an upregulation of MMP-9 in cerebral endothelium and astrocytes (Deb et al, 2003; Lee et al, 2003). When astrocytes are exposed to amyloid together with ApoE, it was observed that ApoE4 isoforms tended to suppress MMP-9 levels (Guo et al, 2006). It is attractive to speculate and hypothesize that differential regulation of MMPs by various ApoE isoforms may in fact alter the balance between a beneficial degradation of amyloid versus too much proteolysis that leads to vascular disruption and hemorrhage.

In summary, whereas the majority of resources are now focused on the neurobiology of Alzheimer's disease, it is worthwhile noting that vascular effects may be extremely important as well (Fig. 4). The major risk factors for sporadic Alzheimer's may be mostly vascular in nature. Amyloid perturbs hemodynamic function and blood flow, and triggers oxidative stress and induces cell death in endothelial cells. Alterations in neurovascular transporters may disrupt the delicate balance between deposition and clearance in aging brain. And amyloid may influence neurogenesis and angiogenesis, in part via modulation of neurovascular proteases that regulate degradation and vessel integrity.

Conclusions and Future Directions

For perhaps far too long, the focus in neurological research was on the neurobiology of CNS disorders. However, numerous failures in clinical trials for "pure" neuroprotectants should now be re-interpreted in the context of the neurovascular unit. A prime example can be found in the use of tPA. Besides dissolving the offending clot, tPA may also trigger a broad spectrum of responses within all compartments of the neurovascular unit. These neurovascular responses must be considered in both acute injury as well as delayed remodeling. Similar arguments may be made for neurodegeneration. Many risk factors for Alzheimer's are linked to vascular function. In transgenic models of Alzheimer's, neurovascular coupling seems to be perturbed prior to neuronal dysfunction, and cerebral endothelial markers of oxidative stress and proteolytic pathology often emerge even before parenchymal plaques appear. Indeed, it is not just abnormal amyloid generation, but also abnormal amyloid clearance and neurovascular kinetics that may contribute to Alzheimer's disease. Ultimately, the endogenous brain response to stress and injury may be mediated by a coordinated coupling between neurogenesis and angiogenesis. Experimental models that probe these altered neurovascular phenomenon may now provide new opportunities for basic and translational research.

The cerebral endothelium may not just comprise inert tubes for blood flow. But instead, endothelium may also be active sources of trophic and signaling agents that subserve neuronal survival and function. Ultimately, we hope that investigations will dissect not just neurobiology, but instead the entire neurovascular unit. Is there a change in neurovascular matrix that mediates signaling between the vascular and neuronal compartments? How do alterations in neurovascular homeostasis affect neuronal function? And in turn, how can neuronal defects perturb vascular regulation? The neurovascular unit provides an integrated framework for hypothesis-testing of function and dysfunction. Based on these ideas, we hope that the coming years will reveal new targets and therapeutic approaches for brain injury, neurodegeneration, and recovery.

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