Dysfunctional Cell-Cell Signaling in the Neurovascular Unit as a Paradigm for Central Nervous System Disease

Shuzhen Guo, PhD; Eng H. Lo, PhD

Abstract—The fundamental premise of neuroprotection has historically focused on the prevention of neuronal death. However, despite tremendous advances in the molecular biology of intraneuronal mechanisms and pathways, a clinically effective neuroprotectant does not yet exist. This problem is especially clear for stroke, for which a large number of neuroprotection trials have failed. The concept of the neurovascular unit emphasizes that cell-cell signaling among the various neuronal, glial, and vascular compartments underlies the homeostasis of normal brain function. Conversely, dysfunctional signaling within the neurovascular unit should contribute to disease. This minireview surveys recent data that support this basic idea, with examples drawn from experimental models broadly relevant to stroke and neurodegeneration. (Stroke. 2009;40[part 2]:000-000.)

Key Words: neuroprotection ■ cell-cell signaling

The “neurovascular unit” is an emerging concept that emphasizes the interactions among different types of cells in the brain, including neuronal, glial, and vascular elements.¹–⁶ Homeostatic signaling within the neurovascular unit underlies normal brain function. Hemodynamic coupling between neurons and the cerebrovasculature mediates the cerebral blood flow response to neuronal activation.⁷ Cell-cell interactions between astrocytes and endothelial cells sustain the functionality of the blood-brain barrier (BBB).⁸ Neurotransmitter trafficking between neuronal release and glial reuptake modulates the kinetics of neurotransmission.⁹¹⁰ Critical interactions between the cerebral endothelium and neural precursor cells provide the “neurovascular niche” as an essential microenvironment for neurogenesis.¹¹¹² At the molecular level, an emerging number of growth factors are now recognized to have dual functions on both endothelial cells and neurons; these mediators are termed “angioneurins.”¹³

If ordered and coupled signaling within the neurovascular unit underlies normal brain function, it is reasonable to postulate that disordered and uncoupled signaling might mediate central nervous system (CNS) disease. To support this hypothesis, this minireview surveys recent data from a range of experimental models of CNS disorders.

Dysfunctional Signaling Between Neurons and Glia

Astrocytes play an essential role in normal brain function. In many neurodegenerative diseases, there are increased astrocyte numbers as well as enhanced glial fibrillary acidic protein immunoreactivity. Insofar as astrocytes play a central role in maintaining glutamate neurotransmitter homeostasis, perturbations in astrocytic glutamate transfer systems, including GLT1 in rodent animal models and EAAT2 in humans, might lead to enhanced excitotoxicity in vulnerable neuronal populations.¹³–¹⁵ Augmented excitotoxicity might then contribute to the pathophysiology of not only stroke but also other CNS diseases.

Huntingtin (htt) is an important genetic factor for Huntington disease (HD). However, transgenic mice expressing mutant htt only in cortical or striatal neurons do not exhibit neurodegeneration, suggesting that cell-cell interactions play a critical role in HD pathology.¹⁶¹⁷ Although mutant huntingtin protein is known to aggregate in the neurons of HD transgenic mouse models, a recent report suggests that it is also present in astrocytic nuclei, a phenomenon that becomes more prominent with age and furthermore corresponds to a downregulation of glutamate transporters in these cells. In neuron-glia cocultures, wild-type glial cells protected neurons against mutant huntingtin-mediated neurotoxicity, whereas glial cells expressing mutant huntingtin increased neuronal vulnerability.¹⁸ Recent human studies may be consistent with these mouse and cell-based observations. Positron emission tomography studies of the molar ratio of cerebral oxygen metabolism to cerebral glucose metabolism (CMRO₂/CMRglc) in early, genetically proven HD patients found increased striatal CMRO₂/CMRglc with unchanged CMRO₂ compared with age-matched control subjects. Because glycolytic metabolism is predominantly astrocytic, the selective reduction in striatal CMRglc suggests that astrocytes may be dysfunctional in HD.¹⁹ Taken together, these observations indicate
that mutant huntingtin in glial cells can contribute to neuronal dysfunction and excitotoxicity in HD brains.

In a similar way, studies of chimeric mice suggest that noncell autonomous processes contribute to motor neuron death in amyotrophic lateral sclerosis (ALS). Familial ALS is associated with mutations in SOD1, the gene that codes for copper-zinc superoxide dismutase. In animals bearing both wild-type cells and cells harboring the SOD1 \(^{G93A}\) transgene, wild-type neurons surrounded by transgenic nonneuronal cells acquired phenotypes characteristic of ALS. Conversely, transgenic neurons associated with wild-type cells were increasingly spared. Results from coculture systems have shown that in fact, fibroblasts, microglia, cortical neurons, and myocytes, release factors selectively toxic to motor neurons. \(^{21}\) mSOD1 \(^{G93A}\) microglia were more toxic to primary neurons in coculture experiments compared with wild-type microglia, with an elevated production of superoxide and nitrite+nitrate. \(^{22}\) In motor neurons derived from embryonic stem cells, cocultures of wild-type motor neurons with glia from SOD1 \(^{G93A}\) mice led to a marked reduction in motor neuron survival. \(^{23}\) Rodent astrocytes expressing mutant SOD1 kill spinal primary and embryonic stem cell--derived motor neurons, apparently triggered by soluble toxic factors acting via a Bax-dependent mechanism. These unknown toxic factors were specific for motor neurons and did not kill spinal \(\gamma\)-aminobutyric acidergic neurons, dorsal root ganglion neurons, or embryonic stem cell derived--interneurons. The importance and specificity of these neuron-astrocyte interactions were confirmed by studies showing that in fact, fibroblasts, microglia, cortical neurons, and myocytes expressing mutant SOD1 do not cause overt neurotoxicity. \(^{24}\)

The homeostatic need for neuron-astrocyte signaling can also be detected in Niemann-Pick disease type C (NPC), a deadly neurodegenerative disease caused by mutations in the gene NPC1. NPC1 \(^{-/-}\) is expressed predominantly in the perisynaptic astrocytic glial processes. \(^{25}\) NPC patients typically show a loss of cerebellar Purkinje cells. However, stereologic counting has also revealed that glia in the corpus callosum are greatly reduced, even early in life. \(^{26}\) Thus, it is possible that glia with NPC mutations can contribute to neurodegeneration. Wild-type neurons cultured on a layer of NPC \(^{-/-}\) astrocytes showed decreased neurite growth compared with those cultured on wild-type astrocytes. A reduced level of estradiol was measured from both astrocyte culture medium and whole brains from NPC \(^{-/-}\) mice. Furthermore, administration of 17\(\beta\)-estradiol to neonatal NPC \(^{-/-}\) mice significantly delayed the onset of neurologic symptoms, increased Purkinje cell survival, and extended the animal’s life span. \(^{27}\) Another group recently reported that replacement expression of NPC1 in astrocytes only by the glial fibrillary acidic protein promoter could ameliorate the number of degenerating neurons and activated astrocytes and extend the overall lifespan of NPC1 \(^{-/-}\) mice. \(^{28}\)

Taken together, these emerging findings in a broad range of CNS diseases all point to common mechanisms relevant to stroke. Neuron-astrocyte interactions are essential to cerebral function. When these signaling connections are dysfunctional or disrupted, neurodegeneration follows. Protecting the glia should be an integral part of any neuroprotective strategy.

### Dysfunctional Signaling Between Neurons and Endothelium

Endothelial cells, together with astrocytes and pericytes, form the BBB. Compromised BBB function is a common occurrence in neurodegenerative disease. \(^{29,30}\) In the context of stroke, damage to the BBB mediates edema and hemorrhage. However, beyond outright disruptions of this barrier, other perturbations of endothelial signaling may also affect neuronal function and survival.

Focal BBB disruption induces in vivo degeneration of dopaminergic neurons, a characteristic of Parkinson disease. A single intranigral injection of vascular endothelial growth factor disrupted the BBB in the ventral mesencephalon and induced tissue responses with histopathologic features of Parkinson disease, ie, loss of tyrosine hydroxylase--positive neurons with concomitant apoptosis andactivation of microglia. Alterations in BBB function may therefore contribute to the pathology of Parkinson disease. \(^{31}\)

Similar endothelial substrates might be involved in Alzheimer disease (AD). Blood vessels isolated from the brains of AD patients can directly kill neurons in vitro in a dose- and time-dependent manner, by either direct coculture or (indirect) exposure to conditioned media from those vessels. In contrast, vessels from non-AD donors, both young and old, are not neurotoxic. Although the vascular factor responsible for this phenomenon has not been unequivocally identified, it appears to be relatively neurospecific, killing primary cortical neurons, cerebellar granule neurons, and differentiated PC-12 cells but not nonneuronal cell types, such as glial cells, other endothelial cells, fibroblasts or smooth muscle cells, or undifferentiated PC-12 cells. \(^{32}\)

Beyond neurotoxicity, the cerebrovasculature may also interact with the pathophysiology of AD via impairments in amyloid transport. \(^{33}\) Accumulating evidence from patients and animal models of AD suggests that vulnerable brains may suffer from an increase in influx receptors (eg, the receptor for advanced glycation end products) and a decrease in efflux receptors (eg, lipoprotein receptor--related protein). Multiple pathogenic cascades originating from altered cerebral arterioles or capillaries may lead to aberrant angiogenesis, cerebral amyloid angiopathy, senescence, and faulty clearance of amyloid across the BBB, thus resulting in increased amyloid-\(\beta\) levels. These neurovascular events may ultimately amplify neuronal dysfunction and injury in AD.

Another neurodegenerative disorder in which vascular compromise may play a role is ALS. The importance of the endothelium was first suggested by an elegant genetic study in which ALS patients as well as the SOD mutant mouse model of ALS showed reduced levels of vascular endothelial growth factor, \(^{34}\) and restoration of vascular endothelial growth factor protected motor neurons from cell death. \(^{35,36}\) More recently, a leaky blood–spinal cord barrier has been detected in early and late symptomatic SOD mutant mice, with downregulation of Glut-1 and CD114 expression in the endothelial cells of the blood–spinal cord barrier. \(^{37}\)

Mechanistically, these phenomena have been shown to be related to alterations in endothelial tight-junction proteins, such as ZO-1, occludin, and claudin-5, which result in microhemorrhages and hypoperfusion that appear to precede...
motor neuron degeneration and the development of neuroinflammation.\(^{38}\)

Dysfunctional signaling between the endothelium and neurons implies that under normal conditions, functional homeostasis exists. If true, this paradigm would suggest that the cerebrovasculature comprises much more than just inert plumbing for the brain. Perhaps the endothelium might even be thought of as a neuroprotective organ embedded within the brain itself. Conditioned media from cerebral endothelial cells broadly protected neurons against amyloid neurotoxicity, endoplasmic reticulum stress, hypoxia, and oxygen-glucose deprivation.\(^{39}\) This phenomenon appeared to be largely mediated by endothelially produced growth factors, such as brain-derived neurotrophic factor, which were sustained by integrin signaling. Nonthyotoxic levels of oxidative stress disrupted integrin function and reduced endothelial levels of neuroprotective factors. These data suggest that the cerebral endothelium provides a critical source of homeostatic support for neurons through matrix and trophic coupling and that a disturbance in this coupling pathway might contribute to neuronal injury.\(^{39}\) These pathways might be relevant not only in stroke but also in vascular dementia and perhaps even other forms of chronic neurodegeneration.

**Conclusions**

Historically, the study of CNS diseases has focused on intraneuronal mechanisms. In recent years, this neuron-based autonomous model has perceptibly shifted to a more integrative paradigm that emphasizes cell-cell interactions. In this minireview, we have surveyed examples from a wide range of disease models wherein signaling perturbations within the neurovascular unit comprise potential mechanisms for neuronal dysfunction and injury. Although we have mostly discussed astrocyte and endothelial interactions, other cell types in the brain, such as microglia, oligodendrocytes and pericytes, will surely play critical roles as well. Protecting neurons alone may not work for stroke and neurodegeneration. The integrity of interactions within the entire neurovascular unit must be rescued. As the network of interacting cells and signals grows, a systems biology approach may eventually be required.

**Acknowledgments**

We gratefully thank Drs Xiaoying Wang, Klaus van Leyen, Ken Arai, Woo Jean Kim, MingMing Ning, and many other colleagues for many helpful discussions.

**Sources of Funding**

This study was supported in part by grants from the National Institute of Neurological Disorders and Stroke and the American Heart Association.

**Disclosures**

None.

**References**


Dysfunctional Cell-Cell Signaling in the Neurovascular Unit as a Paradigm for Central Nervous System Disease
Shuzhen Guo and Eng H. Lo

Stroke. published online December 8, 2008;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2008/12/08/STROKEAHA.108.534388.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/