

## VEGF AND SEIZURES: CROSS-TALK BETWEEN ENDOTHELIAL AND NEURONAL ENVIRONMENTS

### Vascular Endothelial Growth Factor (VEGF) in Seizures: A Double-Edged Sword

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Vascular endothelial growth factor (VEGF) is a vascular growth factor that induces angiogenesis (the development of new blood vessels), vascular permeability, and inflammation. In brain, receptors for VEGF have been localized to vascular endothelium, neurons, and glia. VEGF is upregulated after hypoxic injury to the brain, which can occur during cerebral ischemia or high-altitude edema, and has been implicated in the blood–brain barrier breakdown associated with these conditions. Given its recently described role as an inflammatory mediator, VEGF also could contribute to the inflammatory responses observed in cerebral ischemia. After seizures, blood–brain barrier breakdown and inflammation also is observed in brain, albeit on a lower scale than that observed after stroke. Recent

evidence has suggested a role for inflammation in seizure disorders. We have described striking increases in VEGF protein in both neurons and glia after pilocarpine-induced status epilepticus in the brain. Increases in VEGF could contribute to the blood–brain barrier breakdown and inflammation observed after seizures. However, VEGF also has been shown to be neuroprotective across several experimental paradigms and thus could potentially protect vulnerable cells from damage associated with seizures. Therefore the role of VEGF after seizures could be either protective or destructive. Although only further research will determine the exact nature of the role of VEGF after seizures, preliminary data indicate that VEGF plays a protective role after seizures.

### VEGF-Mediated Inflammation Precedes Angiogenesis in Adult Brain

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Vascular endothelial growth factor (VEGF) has been shown to induce angiogenesis when infused continuously into adult rat brain tissue. In addition, VEGF has been shown to enhance permeability in brain vasculature. Adult rats were continuously infused with mouse VEGF into neocortex for up to 7 days. We studied the development of VEGF-induced vasculature in rat neocortex and evaluated the temporal expression of a wide variety of markers for inflammation and vascular leak in relation to the angiogenic response by using immunohistochemistry and Western blot analysis. We report here that VEGF-mediated inflammation in brain is characterized by upregulation of intercellular ad-

hesion molecule (ICAM)-1 and the chemokine macrophage inflammatory protein (MIP)-1 $\alpha$ , as well as a preferential extravasation of monocytes. VEGF causes a dramatic breakdown of the blood–brain barrier, which is characterized by decreased investment of the vasculature with astroglial endfeet. Perivascular cells, in contrast, increase around the newly formed cerebrovasculature. In addition, breakdown of the blood–brain barrier, leukocyte extravasation, and extracellular matrix deposition occur before vascular proliferation. Furthermore, administration of low doses of VEGF induces permeability and inflammation without appreciable vascular proliferation.

### COMMENTARY

Vascular endothelial growth factor (VEGF) is a key signal in the induction of vessel growth, a process termed

*angiogenesis* (1). VEGF also can alter the blood–brain barrier (BBB) permeability and enhance monocyte infiltration in brain parenchyma (2,3). VEGF is a hypoxia-inducible protein, which promotes its effect on brain microvasculature by acting on

receptor tyrosine kinases on endothelial cells. However, recent studies clearly show that VEGF is not an endothelial cell-specific factor, as it can be expressed by neuroglia or neurons and affect neuronal functions. VEGF may act as a neurotrophic factor and can produce neurogenic effects on neuronal progenitors. In this regard, an elegant study by Cao et al. recently reported that neuronal expression of VEGF in the rat hippocampus enhances neurogenesis and that the phenomenon is associated with improved cognition, independent of endothelial cell proliferation (4).

VEGF also has been suggested as a critical player in neurodegeneration (5). For example, growing evidence exists that insufficient neuroprotection by VEGF may play a role in motoneuron degeneration, and administration of VEGF has favorable effects on recovery from ischemic brain insult by reducing edema formation and infarct volume. Part of this neuroprotective effect may be mediated by its angiogenic properties, leading to improved vascularization and blood perfusion in the diseased tissue. However, other studies suggest that ischemic brain injury may indeed be reduced by inactivation of endogenous VEGF and that VEGF also may produce detrimental effects by inducing vascular leakage, resulting in hemorrhagic transformation of the ischemic lesion. It is, therefore, increasingly apparent that VEGF may subserve multiple roles in the central nervous system (CNS) and peripheral nervous system (PNS). The neuroprotective effects of VEGF could occur independent of a vascular response and be mediated by VEGF receptors directly localized on neurons and glia. Although the primary receptors for VEGF, VEGF receptor 1 (VEGFR1) and VEGF receptor 2 (VEGFR2), both are preferentially localized on the vascular endothelium, VEGFR2 also has been reported on glia and neurons where they might be upregulated during neuronal perturbations. Members of the neuropilin family also can bind VEGF. The neuropilins and their ligands are known to play prominent roles in axonal pathfinding, fasciculation, and blood vessel formation during PNS development.

The neurotrophic and neurogenic properties of VEGF, as well as VEGF effects on angiogenesis and BBB breakdown, have primed interest in its possible involvement in the pathologic events triggered in CNS by seizures, as addressed in the Croll, Goodman, and Scharfman article. These authors reported that seizures, induced by pilocarpine in experimental animals, dramatically increase VEGF both in neurons and in glia. Presumably, VEGF secreted by neurons and glia in epileptic conditions (by binding to VEGFR1 on local microvasculature) may mediate increases in vascular permeability and leakage of the BBB.

The effects of VEGF, and related inflammatory molecules, on BBB permeability are particularly interesting in view of a recent report showing that focal cortical opening of BBB, for at least 6 days, leads to increased excitability and spontaneous paroxysmal events in the surrounding tissue, which is mani-

fested only after a latency of 4 days and lasts for at least 49 days (6). This finding suggests that alterations in BBB permeability per se may be epileptogenic, and the concept is particularly important to the development of acquired epilepsy in humans. It is noteworthy that different insults, resulting in delayed seizures, are all associated with inflammatory reactions in CNS and BBB opening.

The trigger for the induction of VEGF after seizures is still unknown; one possibility is that hypoxia, which is the best-known stimulus for VEGF increase, may play a role. Alternatively, VEGF may be induced by proinflammatory cytokines, such as interleukin (IL)-1 $\beta$ , which are known to increase VEGF in microvasculature (7). IL-1 $\beta$  is rapidly produced and released by glia during seizures (8), and its receptors are localized on glia, neurons, and endothelial cells of the BBB, as well as on monocytes infiltrating the brain parenchyma. Therefore in determining what trigger leads to the production of VEGF in brain under pathologic conditions, the IL-1 $\beta$  cytokine is a good candidate. The available evidence does not yet allow any definite conclusions to be drawn regarding the functional consequences of VEGF production in CNS during seizure activity. However, it appears clear from the work by Croll et al. that the angiogenic effects of the VEGF protein occur at concentrations higher than those required for affecting BBB permeability and inducing inflammatory reactions.

VEGF also acts as a potent proinflammatory cytokine. According to Croll and colleagues, the primary inflammatory reactions induced by VEGF in brain include monocytic infiltrate. The phenomenon is likely a consequence of a direct chemoattractant effect of VEGF, acting on VEGFR1 located on monocytes, or of VEGF-induced upregulation of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ), located in endothelial and brain parenchymal cells. Compelling evidence suggests that proinflammatory cytokines, such as IL-1 $\beta$ , increase seizure activity in rodents (8–10), suggesting that the induction of VEGF (which may be subsequent to IL-1 $\beta$  production and release) could be part of the molecular cascade of events leading to exacerbation of seizures.

In contrast to the possible detrimental effects of VEGF, evidence of a neuroprotective role for VEGF is accumulating. Under conditions of hypoxic, excitotoxic, or oxidative stress, all of which may occur during seizure activity, VEGF increases the survival of hippocampal, cortical, and cerebellar neurons as well as the proliferation of astrocytes and microglia (5). Croll et al. provided evidence for a neuroprotective role in vivo when they showed that continuous hippocampal infusion of VEGF for several days before status epilepticus induction results in sparing of CA1 and CA3 pyramidal cells, which are otherwise highly sensitive to the damaging effects of seizures. The mechanisms of neuroprotection are still hypothetical and may involve

either direct effects of VEGF on neuronal VEGFR2 receptors or indirect effects mediated by astrocytes or microglia.

Therefore, as stated by Croll et al., one can conclude that VEGF is a double-edged sword, a characteristic almost invariably shown by proinflammatory molecules. Thus the final functional outcome of inflammatory reactions in brain may depend on various factors, including the amount of time that the tissue is exposed to these inflammatory molecules, their concentration in brain tissue at the relevant sites of action, and the status of receptor subtypes during the disease process. Considering that some aspects of inflammatory reactions, even in pathologic conditions, may be protective, the major goal should be to learn how to control the complicated inflammatory system to facilitate brain-repair functions and inhibit its detrimental effects.

by *Annamaria Vezzani, Ph.D.*

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