

# VEGF AS A TARGET FOR NEUROPROTECTION

**Vascular Endothelial Growth Factor Is Up-Regulated after Status Epilepticus and Protects against Seizure-Induced Neuronal Loss in Hippocampus.** Nicoletti JN, Shah SK, McCloskey DP, Goodman JH, Elkady A, Atassi H, Hylton D, Rudge JS, Scharfman HE, Croll SD. *Neuroscience* 2008;151(1):232–241. Vascular endothelial growth factor (VEGF) is a protein factor which has been found to play a significant role in both normal and pathological states. Its role as an angiogenic factor is well-established. More recently, VEGF has been shown to protect neurons from cell death both in vivo and in vitro. While VEGF's potential as a protective factor has been demonstrated in hypoxia-ischemia, in vitro excitotoxicity, and motor neuron degeneration, its role in seizure-induced cell loss has received little attention. A potential role in seizures is suggested by Newton et al.'s [Newton SS, Collier EF, Hunsberger J, Adams D, Terwilliger R, Selvanayagam E, Duman RS (2003) Gene profile of electroconvulsive seizures: Induction of neurotrophic and angiogenic factors. *J Neurosci* 23:10841–10851] finding that VEGF mRNA increases in areas of the brain that are susceptible to cell loss after electroconvulsive-shock induced seizures. Because a linear relationship does not always exist between expression of mRNA and protein, we investigated whether VEGF protein expression increased after pilocarpine-induced status epilepticus. In addition, we administered exogenous VEGF in one experiment and blocked endogenous VEGF in another to determine whether VEGF exerts a neuroprotective effect against status epilepticus-induced cell loss in one vulnerable brain region, the rat hippocampus. Our data revealed that VEGF is dramatically up-regulated in neurons and glia in hippocampus, thalamus, amygdala, and neocortex 24 h after status epilepticus. VEGF induced significant preservation of hippocampal neurons, suggesting that VEGF may play a neuroprotective role following status epilepticus.

## COMMENTARY

Vascular endothelial growth factor (VEGF) is a vascular growth factor that induces angiogenesis, increases vascular permeability, and promotes inflammation in the CNS (1,2). VEGF, originally considered as an endothelial-specific growth factor and a potent mitogen for endothelial cells derived from arteries, veins, and lymphatics, has recently been shown

to have direct effects on different cell types, including neurons, Schwann cells, astrocytes, neural stem cells, and microglia. Increased levels of VEGF in the brain have been measured after a variety of insults, including hypoxia/ischaemia and seizures. In particular, following seizure induction in experimental models, VEGF was expressed mainly by neurons and astrocytes (3; Nicoletti et al.), while in human temporal lobe epilepsy specimens, prominent VEGF immunostaining was found also in the microvasculature. The identification of VEGF receptors in epileptogenic tissue—not only on endothelial cells of blood vessels (3) but also on astrocytes and neurons (1,2,4)—raised

the question of the functional consequences of seizure-induced increases in the brain level of VEGF (other than those well described on brain vessels). Neurotrophic and neuroprotective effects of VEGF have been reported in several *in vitro* and *in vivo* experimental conditions, suggesting the possibility that this protein may afford neuroprotection by acting directly on neuronal receptors. Indeed, VEGF receptors appear to be inducible in neurons in pathological states, such as after the induction of status epilepticus (4).

Nicoletti et al. provided evidence that VEGF is strongly upregulated in neurons and glia 24 hours after pilocarpine-induced status epilepticus and established (by pharmacological approaches) that VEGF has a neuroprotective potential against status epilepticus-induced cell loss. Using ELISA, these authors demonstrated that the level of this protein doubled in the hippocampus and cortex of rats exposed to status epilepticus; immunocytochemistry clearly showed that VEGF was upregulated in surviving neurons (the activation resolved by 7 days after status epilepticus) and in activated astrocytes. Increased VEGF expression was observed in all brain regions involved in seizure spread as well as in the associated neuronal cell loss and glia activation. Previous work also reported that the seizure-induced neuronal expression of VEGF is transient, while the astrocytic expression is still evident during epileptogenesis preceding the onset of spontaneous seizures and in chronic epileptic tissue (3).

Nicoletti et al. adopted a pharmacological approach to address the functional meaning of VEGF upregulation following seizures: they chronically infused the hippocampus with the VEGF blocker Fit-Fc (an immunoadhesin designed to sequester VEGF) at a dose known to interfere with endogenous VEGF binding or with human recombinant VEGF at a dose below the doses that are optimal for inducing angiogenesis. Control rats also were assessed, using inactivated VEGF or bovine serum albumin to control for protein load or Fc domain of human IgG (hFc), a recombinant human control protein. After 5 days of protein infusion, rats were exposed to status epilepticus and then killed after 24 hours to evaluate the degree of cell loss. Protein infusion was stopped at the time of killing. Stereological estimates of neuronal density in the infused hippocampus showed a significant increase of pyramidal neuron death in the rats receiving the VEGF blocker, while the rats receiving VEGF had less neuronal loss. Interestingly, the levels of VEGF reached in the hippocampus by this pharmacological treatment were almost 200 times higher than the endogenous increase in VEGF induced by seizures, making it unlikely that the much smaller endogenous increases in VEGF are sufficient to mediate neuroprotection. These neuroprotective effects of VEGF were observed at concentrations that were neither associated with increased vascular density or diameter nor with increased vascular permeability.

Available data suggest that VEGF is endowed with anticonvulsant effects, raising the possibility that its neuroprotective action is mediated by antiictal properties (5). Nicoletti et al., however, reported no apparent changes in motor seizure behavior during status epilepticus in rats that received VEGF or its blocker. Nevertheless, this issue requires further investigation, perhaps by using EEG recording of seizures to unequivocally demonstrate that the VEGF neuroprotection is not a consequence of reduced seizure activity. The molecular mechanisms underlying the neuroprotective effect of VEGF are still mostly unexplored; however, there is evidence that the activation of the VEGF receptor, VEGFR2 (which is overexpressed by neurons following seizures), triggers an intracellular phosphatidylinositol 3-kinase/Akt signaling pathway and inhibition of caspase-3 activity that mediate cell survival (6).

In conclusion, Nicoletti et al. envisaged that small molecules, penetrating the blood–brain barrier and mimicking VEGF neuroprotective effects, might be considered a means to provide cell protection in epilepsy. However, this attractive possibility must take into account that the protein can also provoke effects in brain tissue, such as alterations in blood–brain barrier permeability properties, increased vessel density, and inflammation, that have the potential to promote epileptogenesis (3,7–10). Therefore, a major goal would be to learn how to control the detrimental effects of VEGF and to facilitate its brain repair functions.

by Annamaria Vezzani, PhD

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