**Summary**

We previously reported that blood–brain barrier (BBB) disruption was associated with a pathologic angiogenesis in patients with intractable temporal lobe epilepsy (TLE) and in vivo models. This was confirmed by the overexpression of vascular endothelial growth factor (VEGF) in neurons and astrocytes and of its receptor vascular endothelial growth factor-2 (VEGF-R2) (or flk1) in endothelial cells. Using an original in vitro model, we showed that seizures were sufficient to activate the VEGF/VEGF-R2 system, which promotes vascularization and tight junction disassembly. Such a BBB dysfunction was shown to contribute to epileptogenesis. Therefore, we postulate that drugs that target the specific VEGF-R2 pathways involved in permeability are able to repair the BBB, and, therefore, could reduce epileptogenicity.

**Key Words:** Temporal lobe epilepsy, Epileptogenesis, Hippocampus, Angiogenesis, Blood–brain barrier, Tight junctions, Zonula Occludens-1, Permeability, Rat, Vascular endothelial growth factor, VEGF-R2.

**Temporal lobe epilepsy (TLE) is the most common drug-resistant focal epilepsy.** In the last decade, vascular remodeling and blood–brain barrier (BBB) disruption have been shown to participate in epileptogenesis. Indeed, investigations performed on surgical tissue of patients with intractable TLE and relevant rodent models support the hypothesis that BBB dysfunction contributes directly to epileptogenicity. Indeed, the leakage of serum proteins and ions perturbs the neuronal environment and promotes seizures, edema, and inflammatory/immune responses (Eid et al., 2004, 2005; van Vliet et al., 2007; Marcon et al., 2009; Fabene et al., 2010; Ghosh et al., 2010; Ndode-Ekane et al., 2011; Friedman, 2011; Friedman & Dingledine, 2011; Marchi et al., 2011a,b; Tomkins et al., 2011).

The cause of BBB disruption in epileptic focus is still unknown but the following hypotheses incriminate inflammation. Brain inflammation was particularly described in TLE associated with hippocampal sclerosis, neuronal loss, and gliosis, thereby pointing out the deleterious role of cytokines and prostaglandins released by glial cells and neurons (Vezzani et al., 2012). In addition, vascular inflammation was described as an important cause of intractable epilepsy, due to leukocyte extravasation mediated by chemokines (Fabene et al., 2008, 2010; Marchi et al., 2011b). Recently the role of peripheral inflammation was demonstrated in brain excitability via the secretion of cytokines by other organs (Gallic et al., 2012).

However, other factors that disrupt the BBB are present in brain, in vessels, and in peripheral organs. In this review, we focus on the role of the vascular endothelial growth factor (VEGF), which is known to contribute to vascular permeability within the epileptic tissue.

**Major Recent Findings**

**Vascularization and BBB disruption in epileptic tissue**

An increase in vascularization and increase in BBB permeability, leading to the leakage of large serum proteins, were previously observed in the hippocampi surgically removed from patients with intractable TLE, compared to the hippocampus of nonepileptic patients (Fig. 1). In addition, we found that the vascular density was positively correlated with seizure frequency, but not with other parameters, such as patient age, disease duration, or neuronal damages (Rigau et al., 2007), suggesting that...
these vascular changes are a common etiopathologic cause of intractable focal epilepsies. Moreover, we observed degradation of the lamina and of tight junctions (TJs) in endothelial cells (ECs).

### Angiogenic processes

It has been demonstrated that seizures induce the expression of growth factors like the VEGF (Croll et al., 2004). VEGF and the receptor vascular endothelial growth factor-2 (VEGF-R2) promote angiogenesis and vascular permeability through the activation of collagenase, heparinase, plasminogen activators, and matrix metalloproteinases (Yancopoulos et al., 2000; Choi et al., 2003; Pages & Pouyssegur, 2005; Bikfalvi, 2006; Kowanetz & Ferrara, 2006).

Therefore, we investigated the presence of angiogenic factors and their receptors in chronic TLE and during epileptogenesis in rodents. We found an overexpression of the VEGF and of its main receptor VEGF-R2 in intractable focal epilepsies as well as in rodent models (Rigau et al., 2007). We observed that VEGF was overexpressed in neurons and astrocytes, stored in extracellular vesicles, and released by shedding (Schiera et al., 2007; Proia et al., 2008), whereas VEGF-R2 expression was increased in capillaries and neurons. The kinetics of vascularization and permeability were investigated in vivo, showing that seizures induce overexpression and activation of VEGF/VEGF-R2, which, in turn, promotes BBB disruption and vascularization.

### Role of VEGF/VEGF-R2 signaling in BBB dysfunction

VEGF/VEGF-R2 is a complex system with multiple effects: VEGF is a pleiotropic growth factor regulated by hypoxia or inflammation via transcription factors like hypoxia inducible factor-1 (HIF-1), activator protein-1 (AP-1), specificity protein-1 (Sp-1), signals transducers and activators of transcription-3 (STAT3), which are also activated by seizures, whereas the VEGF-R2 receptor tyrosine kinase is also induced by these pathologic conditions (Feng et al., 1997; Gerber et al., 1997; Feng et al., 1999; Choi et al., 2003; Meissner et al., 2009).

The downstream signaling of VEGF-R2 controls significant biological processes involved in vascular permeability and growth. In ECs, VEGF-R2 activates (1) inositol trisphosphate (IP3), endothelial nitric oxide synthase (eNOS), and phospholipase C (PLC), which induce the proteolysis of the basement membrane by MMP-2 and MMP-9; (2) PLC and P42/44-MAPKinase, which promote EC proliferation; (3) Src and phosphoinositide-3-kinase (PI3K) and p38MAP-kinase leading to the disruption of interendothelial junctions and the migration of ECs.

### How to control angiogenesis and target selectively BBB permeability in epilepsies?

To understand the role of VEGF and its receptor signaling in angiogenesis after seizures we used an integrative in vitro model: organotypic hippocampal cultures (OHCs). In this model all the cell types are conserved as well as the interaction between cells. It is worth noting that blood vessels are still present and able to respond to different stimuli (Moser et al., 2003). Seizure-like events induced by kainate in OHCs, showed that epileptiform activity is sufficient to induce rapid VEGF release and VEGF and VEGF-R2 overexpression, followed by vascularization and tight junction disassembly (Fig. 2) (Morin-Brureau et al., 2011).
Therefore, it could be attractive to target VEGF with antiangiogenic drugs to reduce permeability in intractable epilepsies. A similar approach is usually administrated locally in patients with age-related macular degeneration (Campa & Harding, 2011). We treated OHCs with a neutralizing anti-VEGF antibody after kainate-induced seizures, and observed that it fully prevented the vascularization and the disassembly of tight junction proteins (Morin-Brureau et al., 2011) (Fig. 2).

However, some VEGF signaling pathways contribute directly and indirectly to neuron survival and functions (McCloskey et al., 2005; Lee et al., 2006; Holmes et al., 2007; Nicoletti et al., 2008). First, VEGF-R2 expressed by neurons provides directly an autocrine neuroprotection via the antiapoptotic PI3K/Akt pathway. Then, VEGF-R2 expressed by ECs controls the perfusion in response to the high energetic demand of neurons, by two pathways: PKC induces EC proliferation, increasing the vascularization and eNOS, which adapts rapidly the neurovascular coupling.

Therefore, the positive effects of VEGF-R2 signaling on neuronal function need to be taken into account when antiangiogenic drugs are delivered to the central nervous system.

To control angiogenesis without side-effects, we investigated the precise roles of the main VEGF-R2 pathways by testing the effects of selective inhibitors. We confirmed that Akt/PI3k inhibition worsens neurotoxicity, whereas

---

**Figure 2.**

Role of VEGF in seizure-induced BBB alteration in vitro. In absence of neutralizing antibody, the VEGF released after seizures binds VEGF-R2, inducing an increase in vascularization and a downregulation of ZO-1, 24 h later. However, when the anti-VEGF antibody is added in culture media immediately after seizures, VEGF does not bind its receptor, thereby preventing vascularization and ZO-1 downregulation. Scale bar 400 µm or 20 µm.

*Epilepsia © ILAE*
PKC inhibition reduces vascularization. Finally, we found that Src inhibition maintained or restored BBB integrity, without affecting neuronal survival (Morin-Brureau et al., 2011) (Fig. 3).

**Conclusion: Angiogenesis and Epileptogenesis: A Vicious Circle**

These results show that on one side, seizures promote BBB dysfunction by VEGF/VEGF-R2 up-regulation and activation. On the other side, a large body of literature demonstrates that BBB permeability induces seizures or chronic epilepsy, as reported in the introduction. This vicious cycle can be activated in case of acute or chronic vascular anomalies (stroke, hemorrhages, malformations, head trauma) but also by severe seizures, which trigger the release of angiogenic factors.

Nowadays, the huge development of antiangiogenic therapies for cancer offers the possibility to test drugs that could reduce epileptogenesis by preventing or repairing BBB disruption. Therefore, to improve the BBB function we agree that antiangiogenic strategies need to be selective, either by targeting the Src pathway, or by controlling the angiopoietin/Tie2 system.

**Disclosure**

None of the authors has any conflicts of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

**References**


*Epilepsia* 53(Suppl. 6):64–68, 2012


