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Cerebrovascular Remodeling and Epilepsy

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Abstract

The role of the blood-brain barrier (BBB) in epilepsy has evolved from an obstacle for drug brain delivery to an etiological factor contributing to seizures. Recent evidence has shown cerebrovascular angiogenesis and increased BBB permeability in the epileptic *foci* of patients and in experimental models of seizure. The molecular players involved in cerebrovascular remodeling in the epileptic brain are similar to those reported for other brain disorders. The question arises whether pharmacological solutions restoring a proper BBB permeability and preventing dysregulated angiogenesis could be also beneficial in mitigating seizures. We now summarize the available data supporting the role of vascular remodeling and angiogenesis in the epileptic brain, taking into account that the BBB is a multi-cellular structure, reacting to physiological and pathological stimuli. Drugs targeting aberrant angiogenesis could be beneficial in reducing seizure burden when used in combination with available anti-epileptic drugs. We also offer an overview of novel cellular players, such as pericytes, which may participate in cerebrovascular remodeling in the epileptic brain. The possible role of angiogenesis in drug-resistant forms of epilepsy associated with neurovascular dysplasia is discussed. Finally, we speculate on whether the formation of leaky BBB vessels could have an impact on the cerebrovascular rheology and on the physiological mechanisms regulating brain homeostasis.

Keywords

seizures; angiogenesis; blood-brain barrier; VEGF; inflammation

Introduction: Evolution of the Cerebrovascular Hypothesis of Epilepsy

The cerebrovascular hypothesis of epilepsy was originally drafted in the late 18th century, when Cullen hypothesized a link between blood flow deficiency and seizures. In the early 20th century, Bratz, Spielmeyer, and Vogt debated on the contribution of cerebrovascular morphological anomalies to temporal lobe epilepsy. During the 20th century, Lennox and

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Cobb proposed a role for vasospasm in epilepsy whereas Scholz and Jackson confirmed a link between vasoconstriction and seizures. Subsequently, Penfield demonstrated that regional cerebral blood flow and metabolism rate are coupled to neuronal activity (see Ounsted and others 1986).

In recent years, the cerebrovascular hypothesis of epilepsies has been revisited due to accumulating evidence showing that blood-brain barrier (BBB) damage contributes to ictogenesis, epileptogenesis and sustainment of seizures (see Friedman 2011; Janigro 2012; Marchi and others 2012 for review). It is also becoming increasingly evident that remedying a faulty BBB may constitute a complementary approach to treat epileptic neurons. We will now focus on the pathophysiological significance of BBB remodeling induced by seizures and discuss whether drugs targeting aberrant angiogenesis could be used to mitigate seizures.

Cerebrovascular Remodeling in Epilepsy

When studying the cerebrovasculature, the distinction between arterioles, capillaries, and venules is not only semantic but also bears profound functional implications, to be taken into account when dealing with central nervous system (CNS) disorders (Abbott and others 2010). Arterioles, capillaries, and venules are anatomically distinct one from another, differ in diameter and wall thickness, and are characterized by specific endothelial-to-parenchymal cellular interplays. Increasing evidence supports the notion of brain endothelial cells heterogeneity, characterized by variable patterns of gene and protein expression, tightness, and astrocytic coverage (Macdonald and others 2010). This being said, the BBB is the most studied “segment” of the cerebrovasculature. The BBB is constituted by microcapillary endothelial cells enclosed in a perivascular basal lamina and astrocytic endfeet. Astrocytes constitute a bridge to neurons and parenchymal pericytes, the latter contributing to BBB functions. “The BBB supplies the brain with essential nutrients and mediates efflux of many waste products” (Abbott and others 2006). The BBB was initially considered a *static barrier* to the passage of drugs or blood components into the brain, but is now recognized as a factor participating in the pathophysiology of several CNS disorders (Abbott and others 2010; Neuwelt and others 2011; Zlokovic 2008). The BBB is a *dynamic barrier* with rheological (regulation of cerebral blood flow), transport (MDR), metabolic (P450), and immunological (leukocyte trafficking, adhesion molecules) aspects and functions. Moreover, and differently from neurons, BBB cells are not fixed in a post-mitotic state but rather react to various pathological and rheological signals to stimulate angiogenesis, leading to the formation of new microvessels and vascular remodeling (Abbott and others 2010; Bikfalvi 2006; Rigau and others 2007).

A direct, causative link between BBB damage and seizures has been demonstrated clinically (Tomkins and others 2011) and experimentally (Marchi and others 2007; van Vliet and others 2007; Librizzi and others 2012). BBB damage was observed in experimental models of seizures and in brain tissues resected from drug-resistant epileptic subjects. More recent evidence has demonstrated that BBB damage is part of a multifaceted, pathophysiological process affecting the “epileptic” cerebrovasculature. Specifically, a significant increase of vascular density in the hippocampus of patients suffering from drug-refractory temporal lobe epilepsy was reported (Morin-Brureau and others 2011; Nnode-Ekane and others 2010; Rigau and others 2007). The increased vascularization overlapped with the loss of tight junction proteins and leakage of serum proteins into the brain parenchyma (Michalak and others 2012; Rigau and others 2007; van Vliet and others 2007).

The morphological changes observed in the epileptic *foci* suggested the occurrence of angiogenic processes. The latter was confirmed in temporal lobe epilepsy resection by high

levels of vascular endothelial growth factor (VEGF) in neurons and astrocytes and by the over-expression of the receptor *tyrosine kinase* VEGFR-2 on BBB endothelial cells (Rigau and others 2007; see Figs. 1 and 2). The VEGF/VEGFR-2 system functions according to different pathways. VEGF is a pleiotropic growth factor regulated by hypoxia or inflammation *via* transcription factors such as activator protein-1 (AP-1), hypoxia-inducible factor 1 α (Hif α), specificity protein-1 (Sp-1), and signal transducer and activator of transcription 3 (STAT3). These transcription factors and VEGFR-2 are activated under pathological conditions, such as seizures (Bikfalvi 2006; Choi and others 2003; Feng and others 1997; Feng and others 1999; Gerber and others 1997; Kowanetz and Ferrara 2006; Meissner and others 2009; Pages and Pouyssegur 2005; Yancopoulos and others 2000). In addition, the downstream signaling of VEGFR-2 controls vascular permeability and endothelial cell growth. Specifically, VEGFR-2 activates inositol trisphosphate (IP3), endothelial nitric oxide synthase (eNOS), and phospholipase C (PLC), allowing for proteolysis of the vascular basal membrane by matrix metalloproteinases, collagenase, heparinase, and plasminogen (Fig. 3).

Comparable results were found in a rodent model of limbic epilepsy where the BBB was disrupted during epileptogenesis along with an increased microvascular density (Rigau and others 2007; Marcon and others 2009). Furthermore, *in vitro* seizure-like events, as induced by kainic acid, triggered an angiogenic environment, namely the overexpression of VEGF in neurons and astrocytes and the overexpression of VEGFR-2 receptors in the endothelium and neurons. Similar to *in vivo* data, activation of VEGF-VEGFR-2 *in vitro* induced a significant increase of vascular density and tight junction disassembly. During seizures, VEGF is released to reach the BBB microvessels, interacting with endothelial receptor VEGFR-2 in a fashion similar to what is described for other CNS disease (Schiera and others 2007). The role of the VEGF-VEGFR-2 system in BBB damage was confirmed using recombinant VEGF and an anti-VEGF drug (Morin-Brureau and others 2011; Rigau and others 2007).

Targeting Cerebrovascular Remodeling in the Epileptic Brain

The recognition of cerebrovascular mechanisms of seizure has catalyzed the quest for new therapeutics aimed at reducing seizure burden. Novel drugs targeting the damaged BBB and angiogenesis could be used, alone or in combination with available anti-epileptic drugs (AEDs). Recent evidence has suggested that anti-inflammatory drugs targeting the leaky BBB exert anti-seizure effects. The blockade of BBB adhesion molecules or systemic pre-treatment with glucocorticosteroids (GC) reduced seizure onset and burden in a rodent model (Fabene and others 2008; Fabene and others 2010; Marchi and others 2011a). Remarkably, co-administration of GC and AEDs was effective in a cohort of drug-resistant epileptic children where infantile spasms, epileptic syndromes, and Rasmussen encephalopathy were excluded (Granata and others 2009).

More recent evidence has shown that targeting angiogenesis in the epileptic brain could be a viable approach to reduce seizures (Morin-Brureau and others 2011). Currently, anti-VEGF molecules are being tested for glioblastoma and age-related macular degeneration (Campa and Harding 2011). The latter underscores that new anti-angiogenic therapeutic approaches are clinically sound, granted adequate control of side effects. Supporting a role of angiogenesis in epilepsy, anti-VEGF antibody diminished vascularization and tight junction disassembly induced by kainic acid seizures in organotypic hippocampal culture (Morin-Brureau and others 2011). Although over-production of VEGF during seizures has detrimental effects on BBB permeability, VEGF was also demonstrated to contribute to neuron survival (Holmes and others 2007; Lee and others 2006; McCloskey and others 2005; Nicoletti and others 2008). In particular, VEGFR-2 expressed by neurons directly

provides an autocrine neuroprotection via the anti-apoptotic phosphatidylinositol 3-kinase PI3K pathway. Moreover, VEGFR-2 expressed by BBB endothelial cells was demonstrated to be involved in the neurovascular energetic coupling (Kowanetz and Ferrara 2006). The VEGFR2 response follows two distinct pathways: Protein kinase C (PKC) activation, which induces endothelial cells proliferation and vascularization and eNOS activation, which further modulates the neurovascular interplay. Therefore, the beneficial effects of VEGFR-2 signaling on neuronal function should be taken into account when anti-angiogenic drugs are delivered to the CNS. One approach to control angiogenesis circumventing neuronal side effects could be targeting of the Src kinase pathway (Src). Src inhibition maintains or restores the BBB integrity, without affecting neuronal survival (Morin-Brureau and others 2011; Fig. 3). Other angiogenic factors can modify VEGF effects: Angiopoietins and their receptor Tie2 (tyrosine kinase with immunoglobulin and EGF homology domains) control vascular permeability and angiopoietin1 (Ang1) was shown to prevent the effects of VEGF on the vasculature (Gavard and others 2008).

Angiogenesis, Inflammation, and Epilepsy: A Vicious Cycle

Despite evidence produced in the past years, a resilient debate takes place when attempting to establish a causative role for BBB dysfunction in epilepsy (Friedman 2011; Fig. 2). The question was and remains: Do seizures cause BBB dysfunction and remodeling or vice versa? Another great deal of controversy arises when trying to frame the etiological role of “brain-borne” and of “blood-borne” inflammation in determining BBB damage (Fabene and others 2008; Librizzi and others 2012; Marchi and others 2011b; Vezzani and others 2011b; Vezzani and others 2011a). It is plausible to assume that, in the epileptic brain, a *vicious cycle* ensues where BBB dysfunction facilitates seizures and ongoing seizure activity exacerbates BBB damage and angiogenesis, further reiterating seizures (Fig. 2). Moreover, regardless of the “source” of noxious pro-inflammatory signals, re-establishment of the proper BBB functions and impeding abnormal angiogenesis may be generally beneficial to reduce seizure burden. It is currently not clear whether a BBB mechanism of seizures plays a definite role in drug-resistant forms of epilepsy or whether BBB damage and remodeling is an epiphenomenon of seizures. The latter consideration is applicable to other *non*-neuronal events that were demonstrated to occur in response to seizures (e.g., activation of brain glial and microglial cells, leukocyte–BBB interaction; Fabene and others 2010; Vezzani and others 2011b). Moreover, it remains to be experimentally and clinically tested whether novel BBB drugs exert their maximum anti-seizure effects when administered in combination to AEDs (Marchi and others 2012).

When considering possible clinical applications, the administration of anti-angiogenic molecules was proposed to be beneficial immediately after an ischemic stroke to avoid hemorrhagic transformation (Zhang and others 2000). Anti-angiogenic therapy was also proposed for cases of aggressive brain tumors, where conventional therapies are ineffective to slow neoplastic growth. In the case of epileptic disorders, the paroxysmal nature of the disease may represent an additional challenge to by-pass. An anti-angiogenic therapy may need to be tailored for each patient based on AED responsiveness, seizure frequency, and severity in a manner similar to commonly prescribed AED. It is reasonable to hypothesize that inter-ictal or pre-ictal regimens of anti-angiogenic drugs may be the most effective, preventing BBB leakage and seizures. However, these statements are premature, as this field of research has yet to benefit from a comprehensive evaluation of BBB *status* in relation to seizures characteristics and drug resistance.

Blood-Brain Barrier Remodeling and Neurovascular Dysplasia: A Common Etiological Factor in Drug-Resistant Forms of Epilepsy?

Drug-resistant forms of epilepsy are often accompanied with pathological brain substrates. In cases of symptomatic drug-resistant epilepsies, malformations of cortical development (MCD, (Shorvon 2011a, 2011b; Spreafico and Blumcke 2010) represent a common etiological factor underlying seizures. Although aberrant neuronal circuitry was proposed to be the sole cause of hyper-excitability in the malformed brain areas, experimental evidence exists supporting the presence and role for ectopic BBB microvessels (Fan and others 2008; Hallene and others 2006; Marchi and others 2006).

One of the most used models of MCD is based on the pre-natal exposure to methylazoxymethanoic acid (MAM) in rats. MAM induces an ablation of periventricular neurons impeding their differentiation and migration to the developing cortex. The results include, in the adult rodent brain, cerebral heterotopias similar to those observed in humans (Colacitti and others 1998; Colacitti and others 1999) such as ectopic neurons in the periventricular and hippocampal regions and cortical thinning. Accompanying neuronal ectopias is the loss of radial cortical vessels. In addition, BBB microvessels have a tortuous morphology and are leaky to fluorescent tracers (Marchi and others 2006). MAM rats have a lower seizure threshold and seizures elicited in the MAM brain are associated with a greater extent of BBB leakage compared with seizures induced in naïve animals (Chevassus-Au-Louis and others 1998; Chevassus-Au-Louis and others 1999; Tschuluun and others 2011). The fact that the BBB of MAM is intrinsically leaky may contribute to a lower seizure threshold, tilting the blood-brain parenchyma homeostatic equilibrium toward a pro-seizure state. Furthermore, the greater extent of BBB damage observed in response to seizures could reiterate seizures in a fashion that is more severe compared to naïve brains. What remains unclear is the mechanism underlying BBB dysfunction in MCD and the exact role of angiogenesis. Using naïve, *non*-MCD rodents, recent evidence has proposed a role for the VEGF/VEGR-2 pathway in determining BBB leakage during seizures. Whether a similar mechanism applies to the dysplastic BBB is unknown. This could be of direct clinical relevance owing the fact the MCD is often associated with refractory seizures and it is problematic, using only human tissues, to dissociate the importance of inborn BBB lesions from the effects triggered by seizure activity.

Blood-Brain Barrier Remodeling and the Role of Pericytes

Apart from glial and endothelial cells, pericytes also contribute to BBB physiology and integrity (Winkler and others 2011; Zlokovic 2008; Fig. 1). Pericytes are located in the perivascular space of the BBB surrounding the endothelial cells to which they are connected via integrin molecules, connexin 43, and N-cadherin. The function of pericytes was recently reviewed in Winkler and others (2011). In general, pericytes are involved in the maintenance of proper BBB properties and architecture such as the expression and distribution of tight junction proteins, vascular stability, diameter, and blood flow. Studies conducted using mutant mice demonstrated that pericytes deficiency leads to a leaky BBB (Armulik and others 2010; Armulik and others 2011) and that pericytes are required for proper brain angiogenesis (Daneman and others 2010). As pericytes are anatomically placed between endothelial and glial cells, their deficiency could affect both glial and endothelium functions. Evidence exists for a role of pericytes in controlling potassium inward rectifier (Kir 4.1) and aquaporins (AQ-4) expression, also regulating brain homeostasis (Armulik and others 2010).

Diminished pericyte BBB coverage is a pathological event observed in a variety of brain diseases associated with cerebrovascular instability and neurodegeneration (Winkler and

others 2011). Recent data have shown that ischemia induces pericyte dysfunction. Capillary constriction after acute stroke was determined by pericyte contraction, reducing cerebral blood flow (Yemisci and others 2009). Although no concrete evidence is available yet on the role of pericytes in the epileptic brain, a few clues suggest that, in cases of malformation of cortical development, pericyte BBB coverage may be altered (Daneman and others 2010). The latter may represent a mechanism contributing to the formation of ectopic BBB capillaries in malformed brain areas. Whether seizures per se alter pericytes function and BBB coverage remains to be fully elucidated (Fig. 1).

Does Blood-Brain Barrier Remodeling Influence Brain Autoregulation and Drug Penetration?

The brain autoregulates itself, maintaining an optimal cerebral blood flow despite changes in arterial perfusion pressure. Loss of brain autoregulation is associated with several CNS diseases, resulting in an incapability of balancing between CNS metabolic demand and blood oxygen supply. Cerebral perfusion is regulated by different mechanisms depending on vessel location (subcortical vs. parenchymal), type (venous vs. arterial), innervations, and caliber. Whereas at the level of subcortical vessels, the release of neurotransmitters on endothelial or smooth muscle cells plays a major role, in the case of parenchymal microvessels, glial cells were demonstrated to influence vascular tone (Gordon and others 2007; Gordon and others 2008).

In seizure disorders, the sudden and repetitive changes in metabolic demand occurring during inter-ictal to ictal transitions may challenge the cerebral autoregulatory mechanism. The existence of pathologically remodeled, leaky BBB microvessels may also interfere with the autoregulatory signals coming from the upstream cerebral vessels. Formation of brain edema could be facilitated and exacerbated by the concomitance of leaky BBB vessels and deviation from optimal autoregulatory conditions.

Cerebrovascular integrity and tonicity is also regulated by intravascular pressure forces. Specifically, the oncotic pressure exerted by plasma proteins impedes fluid to leak out of the BBB capillaries. The combination of the intra-vascular oncotic forces and hydrostatic pressure, together with the tightness of the BBB endothelium, prevents the formation of vasogenic edema. What remains to be elucidated is whether this symmetry of pressures and concentration gradients is maintained in the epileptic, remodeled BBB microvessels (Janigro 2012). Moreover, loss of BBB tightness, brain edema, and passage of serum proteins into the brain parenchyma could affect the kinetics of drug penetration into the epileptic brain. Whereas significant effort has been devoted to investigating whether BBB multi-drug transporter proteins impede entry of AEDs into the epileptic brain, the fact that the epileptic BBB is leaky has been somehow overlooked. In the case of tight junction loss, or increased BBB permeability in general, extravasation of serum proteins into the brain may reduce the availability of free drug in a fashion that depends on drug lipophilicity (Kimelberg 2004). Bearing in mind that only a few micrometers separate BBB vessels from neurons, it is reasonable to hypothesize that a remodeled leaky BBB, allowing for plasma protein to penetrate into the brain, could influence the brain availability of AED. Restoring BBB integrity could reestablish the optimal blood-to-brain separation and AED distribution, ultimately improving therapeutic effects.

Conclusions

The discovery that vascular remodeling and angiogenesis occur in the epileptic brain further strengthens the concept that epilepsy shares pathological tracts with other CNS diseases. Based on available evidence, BBB damage and angiogenesis can be considered the common

endpoint of pathological *stimuli* initiated in the brain or in the periphery. This opens a great opportunity for new therapeutic interventions, where drugs used for other brain disease could be “borrowed” in the attempt to treat seizures. Whether vascular remodeling and leaky BBB are specific features of drug-resistant forms of seizures remains to be elucidated. It remains to be determined whether targeting aberrant angiogenesis and leaky BBB vessels could constitute a valid approach to improve seizure control in drug-resistant epilepsy. Whether anti-angiogenic drugs should be administered in combination with AEDs remains to be clarified. Nevertheless, the discovery of common inter-disease features is allowing the field of epilepsy research to move forward, expanding the understanding of this disease.

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References

- Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis.* 2010; 37:13–25. [PubMed: 19664713]
- Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci.* 2006; 7:41–53. [PubMed: 16371949]
- Armulik A, Genove G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell.* 2011; 21:193–215. [PubMed: 21839917]
- Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, Niaudet C, et al. Pericytes regulate the blood-brain barrier. *Nature.* 2010; 468:557–61. [PubMed: 20944627]
- Bikfalvi A. Angiogenesis: health and disease. *Ann Oncol.* 2006; 17(Suppl 10):x65–70. [PubMed: 17018754]
- Campa C, Harding SP. Anti-VEGF compounds in the treatment of neovascular age related macular degeneration. *Curr Drug Targets.* 2011; 12:173–81. [PubMed: 20887245]
- Chevassus-Au-Louis N, Ben Ari Y, Vergnes M. Decreased seizure threshold and more rapid rate of kindling in rats with cortical malformation induced by prenatal treatment with methylazoxymethanol. *Brain Res.* 1998; 812:252–5. [PubMed: 9813354]
- Chevassus-Au-Louis N, Jorquera I, Ben Ari Y, Represa A. Abnormal connections in the malformed cortex of rats with prenatal treatment with methylazoxymethanol may support hyperexcitability. *Dev Neurosci.* 1999; 21:385–92. [PubMed: 10575262]
- Choi JS, Kim SY, Park HJ, Cha JH, Choi YS, Kang JE, et al. Upregulation of gp130 and differential activation of STAT and p42/44 MAPK in the rat hippocampus following kainic acid-induced seizures. *Brain Res Mol Brain Res.* 2003; 119:10–8. [PubMed: 14597225]
- Colacitti C, Sancini G, DeBiasi S, Franceschetti S, Caputi A, Frassoni C, et al. Prenatal methylazoxymethanol treatment in rats produces brain abnormalities with morphological similarities to human developmental brain dysgeneses. *J Neuropathol Exp Neurol.* 1999; 58:92–106. [PubMed: 10068317]
- Colacitti C, Sancini G, Franceschetti S, Cattabeni F, Avanzini G, Spreafico R, et al. Altered connections between neocortical and heterotopic areas in methylazoxymethanol-treated rat. *Epilepsy Res.* 1998; 32:49–62. [PubMed: 9761308]
- Daneman R, Zhou L, Kebede AA, Barres BA. Peri-cytes are required for blood-brain barrier integrity during embryogenesis. *Nature.* 2010; 468:562–6. [PubMed: 20944625]
- Fabene PF, Bramanti P, Constantin G. The emerging role for chemokines in epilepsy. *J Neuroimmunol.* 2010; 224:22–7. [PubMed: 20542576]
- Fabene PF, Navarro MG, Martinello M, Rossi B, Merigo F, Ottoboni L, et al. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nat Med.* 2008; 14:1377–83. [PubMed: 19029985]

- Fan QY, Ramakrishna S, Marchi N, Fazio V, Hallene K, Janigro D. Combined effects of prenatal inhibition of vasculogenesis and neurogenesis on rat brain development. *Neurobiol Dis.* 2008; 32:499–509. [PubMed: 18930144]
- Feng Z, Chang RC, Bing G, Hudson P, Tiao N, Jin L, et al. Long-term increase of Sp-1 transcription factors in the hippocampus after kainic acid treatment. *Brain Res Mol Brain Res.* 1999; 69:144–8. [PubMed: 10350646]
- Feng Z, Zhang W, Hudson P, Bing G, Feng W, Hong JS. Characterization of the long-lasting activator protein-1 complex induced by kainic acid treatment. *Brain Res.* 1997; 770:53–9. [PubMed: 9372202]
- Friedman A. Blood-brain barrier dysfunction, status epilepticus, seizures, and epilepsy: a puzzle of a chicken and egg? *Epilepsia.* 2011; 52(Suppl 8):19–20. [PubMed: 21967353]
- Gavard J, Patel V, Gutkind JS. Angiopoietin-1 prevents VEGF-induced endothelial permeability by sequestering Src through mDia. *Dev Cell.* 2008; 14:25–36. [PubMed: 18194650]
- Gerber HP, Condorelli F, Park J, Ferrara N. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J Biol Chem.* 1997; 272:23659–67. [PubMed: 9295307]
- Gordon GR, Choi HB, Rungta RL, Ellis-Davies GC, MacVicar BA. Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature.* 2008; 456:745–9. [PubMed: 18971930]
- Gordon GR, Mulligan SJ, MacVicar BA. Astrocyte control of the cerebrovasculature. *Glia.* 2007; 55:1214–21. [PubMed: 17659528]
- Granata T, Marchi N, Carlton E, Ghosh C, Gonzalez-Martinez J, Alexopoulos AV, et al. Management of the patient with medically refractory epilepsy. *Expert Rev Neurother.* 2009; 9:1791–802. [PubMed: 19951138]
- Hallene KL, Oby E, Lee BJ, Santaguida S, Bassanini S, Cipolla M, et al. Prenatal exposure to thalidomide, altered vasculogenesis, and CNS malformations. *Neuroscience.* 2006; 142:267–83. [PubMed: 16859833]
- Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. *Cell Signal.* 2007; 19:2003–12. [PubMed: 17658244]
- Janigro D. Are you in or out? Leukocyte, ion, and neurotransmitter permeability across the epileptic blood-brain barrier. *Epilepsia.* 2012; 53(Suppl 1):26–34. [PubMed: 22612806]
- Kimelberg HK. Water homeostasis in the brain: basic concepts. *Neuroscience.* 2004; 129:851–60. [PubMed: 15561403]
- Kowanetz M, Ferrara N. Vascular endothelial growth factor signaling pathways: therapeutic perspective. *Clin Cancer Res.* 2006; 12:5018–22. [PubMed: 16951216]
- Lee SH, Chun W, Kong PJ, Han JA, Cho BP, Kwon OY, et al. Sustained activation of Akt by melatonin contributes to the protection against kainic acid-induced neuronal death in hippocampus. *J Pineal Res.* 2006; 40:79–85. [PubMed: 16313502]
- Librizzi L, Noe F, Vezzani A, de Curtis M, Ravizza T. Seizure-induced brain-borne inflammation sustains seizure recurrence and blood-brain barrier damage. *Ann Neurol.* 2012; 72:82–90. [PubMed: 22829270]
- Macdonald JA, Murugesan N, Pachter JS. Endothelial cell heterogeneity of blood-brain barrier gene expression along the cerebral microvasculature. *J Neurosci Res.* 2010; 88:1457–74. [PubMed: 20025060]
- Marchi N, Angelov L, Masaryk T, Fazio V, Granata T, Hernandez N, et al. Seizure-promoting effect of blood-brain barrier disruption. *Epilepsia.* 2007; 48:732–42. [PubMed: 17319915]
- Marchi N, Granata T, Freri E, Ciusani E, Ragona F, Puvenna V, et al. Efficacy of anti-inflammatory therapy in a model of acute seizures and in a population of pediatric drug resistant epileptics. *PLoS One.* 2011a; 6:e18200. [PubMed: 21464890]
- Marchi N, Granata T, Ghosh C, Janigro D. Blood-brain barrier dysfunction and epilepsy: pathophysiologic role and therapeutic approaches. *Epilepsia.* 2012; 10.1111/j.1528-1167.2012.03637.x

- Marchi N, Guiso G, Caccia S, Rizzi M, Gagliardi B, Noe F, et al. Determinants of drug brain uptake in a rat model of seizure-associated malformations of cortical development. *Neurobiol Dis.* 2006; 24:429–42. [PubMed: 17027274]
- Marchi N, Johnson AJ, Puvenna V, Johnson HL, Tierney W, Ghosh C, et al. Modulation of peripheral cytotoxic cells and ictogenesis in a model of seizures. *Epilepsia.* 2011b; 52:1627–34. [PubMed: 21627645]
- Marcon J, Gagliardi B, Balosso S, Maroso M, Noe F, Morin M, et al. Age-dependent vascular changes induced by status epilepticus in rat forebrain: implications for epileptogenesis. *Neurobiol Dis.* 2009; 34:121–32. [PubMed: 19320047]
- McCloskey DP, Croll SD, Scharfman HE. Depression of synaptic transmission by vascular endothelial growth factor in adult rat hippocampus and evidence for increased efficacy after chronic seizures. *J Neurosci.* 2005; 25:8889–97. [PubMed: 16192378]
- Meissner M, Michailidou D, Stein M, Hrgovic I, Kaufmann R, Gille J. Inhibition of Rac1 GTPase downregulates vascular endothelial growth factor receptor-2 expression by suppressing Sp1-dependent DNA binding in human endothelial cells. *Exp Dermatol.* 2009; 18:863–9. [PubMed: 19469899]
- Michalak Z, Lebrun A, Di Miceli M, Rousset MC, Crespel A, Coubes P, et al. IgG leakage may contribute to neuronal dysfunction in drug-refractory epilepsies with blood-brain barrier disruption. *J Neuropathol Exp Neurol.* 2012; 71:826–38. [PubMed: 22878666]
- Morin-Brureau M, Lebrun A, Rousset MC, Fagni L, Bockaert J, de Bock F, et al. Epileptiform activity induces vascular remodeling and zonula occludens 1 downregulation in organotypic hippocampal cultures: role of VEGF signaling pathways. *J Neurosci.* 2011; 31:10677–88. [PubMed: 21775611]
- Ndode-Ekane XE, Hayward N, Grohn O, Pitkanen A. Vascular changes in epilepsy: functional consequences and association with network plasticity in pilocarpine-induced experimental epilepsy. *Neuroscience.* 2010; 166:312–32. [PubMed: 20004712]
- Neuwelt EA, Bauer B, Fahlke C, Fricker G, Iadecola C, Janigro D, et al. Engaging neuroscience to advance translational research in brain barrier biology. *Nat Rev Neurosci.* 2011; 12:169–82. [PubMed: 21331083]
- Nicoletti JN, Shah SK, McCloskey DP, Goodman JH, Elkady A, Atassi H, et al. Vascular endothelial growth factor is up-regulated after status epilepticus and protects against seizure-induced neuronal loss in hippocampus. *Neuroscience.* 2008; 151:232–41. [PubMed: 18065154]
- Ounsted, C.; Lindsay, J.; Richards, P. Temporal lobe epilepsy: a biographical study 1948–1986. London, England: Mac Keith Press; 1986.
- Pages G, Pouyssegur J. Transcriptional regulation of the vascular endothelial growth factor gene—a concert of activating factors. *Cardiovasc Res.* 2005; 65:564–73. [PubMed: 15664382]
- Rigau V, Morin M, Rousset MC, de Bock F, Lebrun A, Coubes P, et al. Angiogenesis is associated with blood-brain barrier permeability in temporal lobe epilepsy. *Brain.* 2007; 130:1942–56. [PubMed: 17533168]
- Schiera G, Proia P, Alberti C, Mineo M, Savettieri G, Di Liegro I. Neurons produce FGF2 and VEGF and secrete them at least in part by shedding extracellular vesicles. *J Cell Mol Med.* 2007; 11:1384–94. [PubMed: 18205708]
- Shorvon SD. The causes of epilepsy: changing concepts of etiology of epilepsy over the past 150 years. *Epilepsia.* 2011a; 52:1033–44. [PubMed: 21480878]
- Shorvon SD. The etiologic classification of epilepsy. *Epilepsia.* 2011b; 52:1052–7. [PubMed: 21449936]
- Spreafico R, Blumcke I. Focal cortical dysplasias: clinical implication of neuropathological classification systems. *Acta Neuropathol.* 2010; 120:359–67. [PubMed: 20607544]
- Tomkins O, Feintuch A, Benifla M, Cohen A, Friedman A, Shelef I. Blood-brain barrier breakdown following traumatic brain injury: a possible role in posttraumatic epilepsy. *Cardiovasc Psychiatry Neurol.* 2011; 2011:765923. [PubMed: 21436875]
- Tschuluun N, Jurgens WH, Doisy ET, Schwartzkroin PA. Initiation of epileptiform activity in a rat model of periventricular nodular heterotopia. *Epilepsia.* 2011; 52:2304–14. [PubMed: 21933177]

- van Vliet EA, da Costa AS, Redeker S, van Schaik R, Aronica E, Gorter JA. Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. *Brain*. 2007; 130:521–34. [PubMed: 17124188]
- Vezzani A, Aronica E, Mazarati A, Pittman QJ. Epilepsy and brain inflammation. *Exp Neurol*. 2011a;10.1016/j.expneurol.2011.09.033
- Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol*. 2011b; 7:31–40. [PubMed: 21135885]
- Winkler EA, Bell RD, Zlokovic BV. Central nervous system pericytes in health and disease. *Nat Neurosci*. 2011; 14:1398–405. [PubMed: 22030551]
- Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature*. 2000; 407:242–8. [PubMed: 11001067]
- Yemisci M, Gursoy-Ozdemir Y, Vural A, Can A, Topalkara K, Dalkara T. Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nat Med*. 2009; 15:1031–7. [PubMed: 19718040]
- Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, Powers C, et al. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. *J Clin Invest*. 2000; 106:829–38. [PubMed: 11018070]
- Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron*. 2008; 57:178–201. [PubMed: 18215617]

Figure 1.

Schematic representation of cerebrovascular angiogenesis. Formation of a new collateral blood-brain barrier (BBB) capillary in response to pathological stimuli is shown. Pro- and anti-angiogenic factors are released, directing endothelial cells (EC), pericytes, basal membrane, and tight junctions (TJs) functions. We propose a loss of pericytic coverage upon exposure to a pathological signal (e.g., seizures). Along with vascular endothelial growth factor (VEGF) signaling, loss of pericytes could play an important role in determining changes of BBB permeability.

Figure 2.

Schematic summary of the players contributing to the *vicious circle* of blood-brain barrier (BBB) damage and ictogenesis. Irrespective of the fact that BBB damage (BBBD) precedes or results from seizures (blue circular arrows), vascular endothelial growth factor (VEGF) is released by neurons and glial cells (yellow dots) along with pro-inflammatory cytokines (m = microglial cells). VEGF acts on the VEGF-R2 receptors expressed on BBB endothelial cells increasing permeability, triggering angiogenesis, and vascular remodeling (see also Fig. 1). The formation of new, leaky BBB vessels is proposed to further promote seizures, reinforcing the *vicious cycles* (blue arrows). VEGF was also demonstrated to have neuroprotective effects (see also Fig. 3). Serum components (black dots) are free to penetrate into the brain in conditions of BBBD, altering the brain *milieu* and promoting seizures. Both brain (glial, microglial activation) and blood inflammation was shown to promote BBBD. A peripheral response encompasses blood leukocyte activation and interaction with the BBB (over-expression of chemokines and adhesion molecules) and the involvement of districts of immune competency (e.g., spleen, bone marrow). Under this condition, release of stress hormones, cytokines, and neurotransmitters into the peripheral blood could also alter BBB integrity and promote seizures.

Figure 3.

Angiogenesis signaling pathways in epilepsy. Proposed vascular endothelial growth factor (VEGF) pro-angiogenic pathways: (i) Inositol trisphosphate (IP3), endothelial nitric oxide synthase (eNOS), and phospholipase C (PLC) induce the proteolysis of the basement membrane by MMP-2 and MMP-9. (ii) PLC and P42/44-MAP kinase promote endothelial cells (EC) proliferation and migration. (iii) Src, PI3K, and p38MAP kinase degrade the tight junctions. VEGF neuroprotective pathways: (i): Local perfusion is improved by PKC-mediated ECs proliferation, whereas eNOS affords a rapid adaptation of local blood flow to neuron energetic demand. (ii) VEGFR-2 expressed by neurons provides an autocrine protection via the anti-apoptotic PI3K pathway. Angiopoietins effects: Ang1, released by pericytes is a constitutive ligand of Tie2, enabling cell maturation and protections via PI3-K/Akt. On the contrary, the inducible Ang2, released by endothelial Weibel–Palade bodies, acts as a Tie2 antagonist worsening VEGF effects on the vasculature. The ratio Ang1/Ang2 has an impact on vascular permeability.