Critical Reviews

The Blood–Brain Barrier and Epilepsy

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Summary: During the past several years, there has been increasing interest in the role of the blood–brain barrier (BBB) in epilepsy. Advances in neuroradiology have enhanced our ability to image and study the human cerebrovasculature, and further developments in the research of metabolic deficiencies linked to seizure disorders (e.g., GLUT1 deficiency), neuroinflammation, and multiple drug resistance to antiepileptic drugs (AEDs) have amplified the significance of the BBB’s relationship to epilepsy. Prior to 1986, BBB research in epilepsy focused on three main areas: ultrastructural studies, brain glucose availability and transport, and clinical uses of AEDs. However, contrast-based imaging techniques and medical procedures such as BBB disruption provided a framework that demonstrated that the BBB could be reversibly disrupted by pathologic or iatrogenic manipulations, with important implications in terms of CNS drug delivery to “multiple drug resistant” brain. This concept of BBB breakdown for therapeutic purposes has also unveiled a previously unrecognized role for BBB failure as a possible etiologic mechanism in epileptogenesis. Finally, a growing body of evidence has shown that inflammatory mechanisms may participate in the pathological changes observed in epileptic brain, with increasing awareness that blood-borne cells or signals may participate in epileptogenesis by virtue of a leaky BBB. In this article we will review the relationships between BBB function and epilepsy. In particular, we will illustrate consensus and divergence between clinical reality and animal studies.

Key Words: Antiepileptic drugs—Membrane transport proteins—Monosaccharide transport proteins—Epileptogenesis—Cerebral blood flow.

Fig. 2 shows the proposed links between BBB and epilepsy. Drug resistance affecting approximately 30% of patients, and the possible role of the BBB, obviously remain an important focus of epilepsy research. Additionally, a compromised BBB has been associated with seizures in a number of disorders. Not only congenital defects, such as Glut1 deficiency, but acquired deficiencies, like those resulting from brain tumors, head trauma, etc., often result in seizure disorders. More recently systemic and immune triggers have been implicated in a leaky BBB and neuroinflammation. Understanding the nature of the role of BBB in these disorders is imperative in the treatment of the disease, but the fundamental question of whether the compromised integrity of the BBB is a component of the etiology of epilepsy or a consequence of seizures remains unanswered.

REFRACTORY EPILEPSY: IS THE BBB INVOLVED?

The BBB is a physical and metabolic barrier which serves to regulate and protect the microenvironment of the brain. Composed of a monolayer of brain capillary endothelial cells, it is distinguished by the presence of tight junctions and a relative paucity of fenestrae and pinocytotic vesicles that restrict brain uptake of
circulating molecules. Thus, the BBB limits access to the brain to small nonpolar molecules by passive diffusion or catalyzed transport of large and/or polar molecules (Pardridge, 1999). ABC efflux transporters at the BBB influence the brain uptake of a variety of therapeutic agents, including many AEDs (Golden and Pollack, 2003).

Permeability of the BBB is one of the factors determining the bioavailability of therapeutic drugs and resistance to chemically different AEDs. The permeability of the BBB becomes particularly relevant in drug resistant patients. There are two main theories describing drug resistance in epilepsy: the target hypothesis and the transporter hypothesis. The target, or pharmacodynamic, hypothesis of pharmacoresistance is based on a modification of the molecules targeted by the AED, thus reducing the efficacy of the drug. Changes in known AED targets include altered subunit expression in sodium channels (Ellerkmann et al., 2003; Whitaker et al., 2001; Aronica et al., 2001; Bartolomei et al., 1997), expression of AED sensitive sodium channels in interneurons (Remy and Beck, 2006; Remy et al., 2003), increased expression of T-type calcium channels (Su et al., 2002; Huguenard, 2002), decrease of GABA\textsubscript{A} receptor \(\alpha_1\) subunits and increase of GABA\textsubscript{A} receptor \(\alpha_4\) subunits (Brooks-Kayal et al., 1998). Recently, the possibility that GABA currents are kinetically altered in drug resistant epileptic brain has been proposed by elegant experiments by Ragozzino et al. (2005). The transporter, or pharmacokinetic, hypothesis suggests that effective concentrations of the AED are not attained in the brain, because of aberrant functioning of multidrug transporters. Changes in drug efflux transporters include the overexpression of P-glycoprotein (MDR1) (Marchi et al., 2004; Aronica et al., 2003; Tishler et al., 1995b), MRP1 (Sisodiya et al., 2002; Sisodiya et al., 2001), MRP2 (Dombrowski et al., 2001), and MVP (van Vliet et al., 2004). Although the issue of the relative contributions of the two hypotheses remains unresolved, the transporter hypothesis emphasizes a predominant role of the BBB, and thus will be our primary focus here.
Human studies

The ABC transporter P-glycoprotein (Pgp or MDR1) recognizes a wide range of substrates, including a number of AEDs, and is thought to play a major role in drug extrusion (Loscher, 2002; Potschka et al., 2002; Rizzi et al., 2002). In addition to Pgp the ABC transporters of the multidrug resistance protein (MRP) family and the breast cancer resistance protein (BCRP) are involved in the regulation of brain uptake and extrusion of drugs (Loscher and Potschka, 2005). MDR1 overexpression has been demonstrated in a variety of cells in both the BBB and the parenchyma in patients with intractable epilepsy (Marchi et al., 2004; Sisodiya et al., 2002b; Tishler et al., 1995a). It has been shown that MDR1 is expressed in the cells that are the very target of its extrusion action, namely the neurons (Marchi et al., 2004; Sisodiya et al., 2002). Paradoxically, while expression of MDR1 at the BBB may lead to decreased brain interstitial levels, the converse is true if neurons and glia extrude drugs from the intra- to the extracellular space. This complex pattern of MDR1 expression in epileptic patients does not directly support a significant pharmacokinetic role in human epilepsy (Marroni et al., 2003; Aronica et al., 2003; Abbott et al., 2002; Golden and Pardridge, 2000). While localization of the drug extrusion pump in the BBB is consistent with the pharmacokinetic explanation for drug resistance, it is still unclear if or how the presence of MDR1 in the parenchyma affects drug delivery and distribution or whether it is involved in other cellular functions. It has been suggested that overexpression of MDR1 leads to functional alterations in the CNS that may be linked not only to drug pharmacokinetics but also neuroglial survival in injured brain (Marchi
therapeutic serum levels (15–34 µM) were achieved. When concentrations of carbamazepine found in multi-
ple drug resistant brain were directly applied to human
cortical slices from drug resistant patients made hy-
perexcitable and hypersynchronous by Mg2+-free me-
dia, bursting frequency was not significantly affected,
but overall excitability was reduced by 40%. Similar re-
sults were obtained for phenytoin. At higher AED con-
centrations (60–200 µM), a dose-dependent decrease of
bursting frequency and amplitude was observed. Slices
from drug resistant epileptic patients made hypersyn-
chronous/hyperexcitable by elevated potassium (Oby
et al., 2006; Leschinger et al., 1993) or inhibition of
GABA-A receptors (Oby et al., 2006; de Feo et al., 1991)
behaved similarly. Of note is the response of slices from
human multiple drug resistant brain, which was greater
than in rodent cortex from naïve animals. Taken together,
these results support the hypothesis that multiple drug re-
sistance to AEDs involves cerebrovascular changes that
impede the achievement of appropriate drug levels in the
central nervous system.

Newly discovered drug transporters, often with prop-
erties similar to MDR1, offer further insight into AED
extrusion and the pharmacokinetics of drug resistant
epilepsy. For example, there is a significant overlap be-
tween molecules transported by MDR1 and the non-ABC
transporter RalA Binding Protein 1 (or RLIP76 (Awasthi et
al., 2002a,b; 2003a,b)), suggesting that MDR1 might not
be the exclusive mechanism responsible for drug efflux
(Awasthi et al., 2002; 2003a,b). In particular, RLIP76 is
important in transporting phenytoin and carbamazepine
at the human BBB, highlighting a potentially signifi-
cant function in determining drug-resistance in epilepsy
(Awasthi et al., 2005). In fact, RLIP76 fulfills many of the
predicted properties for a mediator of CNS pharmacoresis-
tance, including: (1) presence at the anatomical interface
between brain and blood; (2) transport of AEDs; (3) func-
tional expression in brain microvascular endothelial cells
but not in parenchymal glia or neurons; and (4) increased
CNS accumulation of phenytoin in RLIP76−/− mice. The
role of RLIP76 in AED resistance is still in its infancy and
more research is needed to fully evaluate its potential as a
drug resistance candidate.

Even with the increasing information regarding drug
transporters, it is unclear if or how the distribution of
MDR1, MRPs, and RLIP76 is related to the pathology
of epilepsy itself. It is possible that their overexpression is
a response to a hostile environment or that they are regu-
lated exclusively by chemotherapy. Both MDR1 and MRP
are involved in cell survival and evidences have shown
that apoptotic mechanisms are not commonly observed
in epilepsy. Seizures per se do not elicit a consistently
homogenous, model-independent cell death phenotype.
Animal studies generally support necrosis as the prin-
cipal morphological phenotype of dying cells after seizures
(Ebert et al., 2002; Puig and Ferrer, 2002; Kubova et al.,
2001; Fujikawa et al., 2000). True apoptotic morphology
may comprise only a small percentage of seizure-induced
cell death, with the dentate gyrus and the setting of kin-
ding being possible exceptions (Sloviter, 1999). These
findings suggest expression of MDR1 in epileptic brain
may be one of the underlying causes for neuroligical sur-
vival and resilience. Interestingly, the original proposed
role for RLIP76 was also that of a molecule involved in
detoxification or protection of cells living in hostile en-
vironments (Awasthi et al., 2002b; 2003c). It is possible
that expression of multiple drug resistance molecules, co-
operating in drug extrusion, is a consequence of impaired
cellular homeostasis.

Animal studies

A pervasive problem in studying drug resistance is the
lack of brain tissue from patients with drug responsive
epilepsy. So, while increased expression of drug trans-
porters has been reported in brain tissue of patients with
refractory epilepsy, the lack of adequate controls makes
comparison between drug resistant and drug respondent
patients problematic. Thus, the question persists as to
whether the increased drug transporter expression in pa-
tients with drug resistant epilepsy is a cause of phar-
macoresistance, an effect of uncontrolled seizures, or an
epiphenomenon occurring in epileptic brain tissue irre-
spective of drug responsiveness. Although sporadic infor-
mation is available on the specific cellular biochemical
changes that occur during the induction of seizures in an-
imal models of epilepsy, such models may be able to pro-
vide some insight as to the question of cause and effect.

Many researchers have shown that there is an overex-
pression of Pgp in endothelial cells, astrocytes and neurons
in a variety of animal models of epilepsy (Volk et al., 2004;
Lazarowski et al., 2004; Kwan et al., 2002; Rizzi et al.,
2002; Zhang et al., 1999). Seizures have been shown to
induce overexpression of other transporters as well. The
expression pattern of MRPl, MRPl2 and BCRP protein in
chronic epileptic rats is associated with the occurrence of
SE, as well as spontaneous seizure activity, and is most
evident in rats with frequent daily seizures (van Vliet
et al., 2005b).

In many cases the overexpression of efflux drug trans-
porters contributes to reduced efficacy of AEDs. Consis-
tent with a significant MDR1 role in AED resistance is the
fact that mice lacking Pgp display greater central nervous system accumulation of AED (Rizzi et al., 2002; Schinkel et al., 1995). Similarly, pharmacologic inhibition of MRPs increased the concentrations of phenytoin, which were found to be significantly lower in epileptic rats compared to controls (van Vliet et al., 2005a). RLIP-76/−/− animals displayed AED neurotoxicity suggestive of an RLIP-76 dependent component of drug extrusion (Awasthi et al., 2005).

An animal model of epilepsy with spontaneous recurrent seizures in which it is possible to select subgroups which respond to or are resistant to AED treatment provides a distinct opportunity to address some of the lingering questions about the role of active drug transporters in pharmacoresistance (Brandt et al., 2004). Using this model, Volk and Loscher have shown overexpression of Pgp in the brain capillary endothelial cells in limbic brain regions of nonresponders compared to responders (Volk and Loscher, 2005). However, the question of whether the overexpression of Pgp is a cause or a consequence of the resistance could not be addressed with this model.

While seizures seem to be a factor in the overexpression of efflux drug transporters, AEDs themselves have also been shown to have an impact on the expression of MDR1. In astrocyte cell cultures from Wistar rats, common AEDs stimulated the overexpression of Pgp in astrocytes in a dose- and time-dependent manner (Lu et al., 2004).

If the BBB is an impediment to CNS drug delivery, one is tempted to circumvent or disrupt the endothelial layer. This has been attempted for CNS tumors, and recent discoveries are supporting the approach by semi-invasive, chronic methodologies (for review see (Czeisler and Janigro, 2006)). One interesting question relates to the possibility that highly lipophilic drugs (e.g., AEDs) will poorly partition across a leaky BBB due to perivascular edema. If this were indeed the case, the design of more polar molecules may, paradoxically, be a solution.

In conclusion, the hypotheses of mechanisms of drug resistance are not mutually exclusive. In fact, the underlying mechanism of pharmacoresistant epilepsy is probably some combination of alterations of AED targets and transporters. An opening in the BBB would provide better access to the brain parenchyma for AEDs, perhaps in spite of an upregulation of multidrug transporters, but partition with perivascular edema may be a problem.

ALTERED METABOLISM IN EPILEPSY

The BBB has developed a sophisticated mechanism to carry glucose efficiently into the brain. This sodium- and insulin-independent transport depends on endothelial expression of GLUT1 (Cornford et al., 1998; Cornford et al., 1994). GLUT1 mediates glucose transport across the BBB and is thus essential for brain energy metabolism. First described in 1991 (De Vivo et al., 1991), the glucose transporter I deficiency syndrome is attributed to a defect of GLUT1 causing impaired transport of glucose across the BBB, interfering with cerebral energy metabolism and brain function, ultimately leading to seizures (De Vivo et al., 2002; De Vivo et al., 1991). While the resulting seizures do not generally respond to common AEDs, they can be controlled by strict adherence to a ketogenic diet (Withrow, 1980). Understanding the mechanism of action of the ketogenic diet may perhaps provide insight into how other types of seizures can be controlled.

The ketogenic diet changes biochemical parameters of the blood, significantly altering the level of ketone bodies. Ketone bodies are the principal alternative energy source for the brain at times of glucose shortage. They exert an anticonvulsant effect that is maintained as long as the blood ketone bodies are elevated (Morris, 2005). It has been hypothesized that ketone bodies have a direct anticonvulsant effect. In particular this has been shown experimentally for acetone (Likhodii et al., 2003), but the toxicity of this agent must be kept in mind as well. Another possibility is that cerebral ketone body metabolism reduces neuronal excitability by increasing cerebral energy reserves (De Vivo et al., 1978). Less direct effects of the diet could include an influence of ketone bodies on the neurotransmitters glutamate and GABA, or a link between the high levels of ketone bodies and the metabolic and homeostatic constraints seen in epileptic tissue (Janigro, 1999). Regardless of how a ketogenic diet may prevent seizures in GLUT1-deficient children, it is clear that BBB dysfunction is a major etiological event in this subtype of seizure disorders.

Human studies: beyond GLUT1 deficiency

The understanding of the involvement of glucose transporters in seizures resulting from GLUT1 deficiency syndrome has led to the investigation of the properties of GLUT1 in epilepsy. The possibility that the hypometabolic aspect of GLUT1-deficient brain may extend to epileptic cortex in general (Sadzot et al., 1992; Henry et al., 1991) has expanded the relevance of these early studies. Fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) studies of patients with complex partial seizures yielded interictal scans that exhibit hypometabolic regions including the epileptic focus as identified by EEG; in contrast ictal scans showed focal, multifocal and generalized increases in the metabolic rate (Engel et al., 1982). However, ictal hypermetabolism did not always correspond to regions of interictal hypometabolism. More recent studies concluded that in epileptogenic temporal cortex both the physiology of blood flow and glucose metabolism are altered. Cornford hypothesized that the interictal zone of hypometabolism coincides with altered BBB transporter activity and demonstrated that the zone of reduced metabolism according to a FDG-PET corresponds to a region of decreased
BBB glucose transporter activity (Cornford, 1999). Further, the reduced transport appears to be limited to the brain capillary endothelial membranes and not at the membranes of cells in the neuropil. To supplement our understanding of GLUT1 from PET, immunogold analyses of BBB GLUT1 were performed on tissue resected from patients undergoing surgery for seizures or other CNS complications. Observations provided evidence in support of PET analyses that showed GLUT1 downregulation in the endothelial cells in regions within and around the seizure focus (Cornford, 1999).

Animal studies
Despite its proven efficacy in controlling seizures, the ketogenic diet has not until recently been extensively explored in animal models. It has been shown that the diet most effectively protects against clonic-type seizures activity (Bough et al., 2002) by elevating seizure threshold (Rho et al., 1999; Bough and Eagles, 1999; Hori et al., 1997). The anticonvulsant effect of the diet in animals is proportional to ketosis, as measured by blood levels of β-hydroxybutyrate, and the diet changes β-hydroxybutyrate levels in days (Rho et al., 1999; Bough and Eagles, 1999). Other animal studies have exhibited long-term effects on seizure occurrence with a ketogenic diet, suggesting that its mechanism of action is not only anticonvulsant but also antiepileptogenic (Rho et al., 2005; Su et al., 2000; Muller-Schwarze et al., 1999). In a kainic acid model of epilepsy, long-term epileptic consequences, i.e., spontaneous recurrent seizures and mossy fiber sprouting, are reduced with the ketogenic diet (Muller-Schwarze et al., 1999). Both effects indicate a retardation of epileptogenesis. These findings underscore that BBB-related hypometabolism may be a common feature of focal epilepsy and not only limited to molecular changes in GLUT1 transporter expression. This is supported by human studies as discussed above.

Since metabolic dysfunction has been implicated in the pathogenesis of temporal lobe epilepsy, the array of defects originally attributed to GLUT1 deficiency have allowed us to explore a specific manifestation of the ambiguities of the role of the BBB in epilepsy. In the face of uncertainties associated with the disease and the treatment, there are a number of clear directions for future studies. For example, to make more effective use of the ketogenic diet as a line of defense against seizures, we need to further explore its mechanism of action, in particular regarding the role of metabolic diffusion barriers in the epileptic CNS. In addition, the role of metabolic transport at the BBB could offer invaluable insights into seizure properties. In both cases, the use of animal models appears a valuable choice which should not be dismissed as a practical means of investigation. In addition, the availability of “metabolic imaging” has provided us with the unique opportunity to visualize the metabolic defects in the ictal and interictal human brain. Thus, combining imaging studies in animals with clinical studies emerges as the most powerful approach towards the development of a metabolic approach to seizure therapy.

BBB FAILURE IN EPILEPSY: ETIOLOGY VERSUS CONSEQUENCE
There is an indisputable correlation between BBB disruption and seizures. Seizures and epilepsy are commonly observed in conjunction with stroke, traumatic brain injury and central nervous system infections, all conditions known to result in compromised BBB function (Ballabh et al., 2004; Herman, 2002; Tomkins et al., 2001; Salazar et al., 1985). In addition, regional patterns of BBB breakdown have been described during epileptiform seizures induced in animal models by various convulsive agents (Nitsch and Klatzo, 1983). Unfortunately, while several microangiographic models are available to study the BBB in animal models, a suitable (i.e., microscopic, quantitative, and minimally invasive) technique for evaluating BBB integrity in humans does not exist. Compounded by insufficient means of investigation, points of debate are whether (1) the BBB fails before, during, or after seizures; (2) the compromised integrity of the BBB is a component of the etiology of epilepsy or a consequence of seizures; and (3) “BBB drugs” (e.g., steroids or NSAID) can have a beneficial effect (Vezzani and Granata, 2005; Sztriha et al., 1986).

Human studies
BBB disruption after acute head trauma is a well-known pathologic finding in both animal studies and humans (Korn et al., 2005; Baskaya et al., 1997; Chen et al., 1996). This disruption may persist for weeks to years after the injury and may be associated with abnormal EEG activity (Korn et al., 2005). Whether this abnormal activity develops into epilepsy is currently unknown, but observations have suggested BBB disruption in conjunction with a slowing in EEG activity may be a precursor to seizures (Pavlovsky et al., 2005). Others have observed persistent BBB disruption in the absence of any evidence of active epileptic foci (Tomkins et al., 2001).

It has been demonstrated that with relatively severe loss of BBB function there is extravasation of serum albumin into capillary endothelial cells, basal lamina and neuropil (Cornford et al., 1995). In human tissue resected from epileptogenic foci, actively spiking regions are characterized by more extravasation than less actively spiking areas (Cornford et al., 1998). Thus, the BBB integrity is closely correlated to the electrophysiological properties of the tissue as evaluated by intraoperative EEG.

The heightened interest in osmotic opening of the BBB as a viable mechanism of increased drug delivery to the brain (Siegal et al., 2000; Kroll and Neuwelt, 1998; Neuwelt et al., 1986; Neuwelt et al., 1983) provides an opportunity to explore the connection between BBB
disruption and seizures in a more controlled, yet “human” environment. Osmotic opening of the BBB by intracarotid infusion of a hypertonic mannitol solution is mediated by vasodilatation, shrinkage of cerebrovascular endothelial cells and modulation of the contractile state of the endothelial cytoskeleton and junction proteins by increased intracellular calcium, with widening of the interendothelial tight junctions to an estimated radius of 200 Å. The marked increase in apparent BBB permeability to intravascular substances (10-fold for small molecules) following the osmotic procedure is due to both increased diffusion and bulk fluid flow across the tight junctions. The permeability effect is largely reversed within minutes to hours (Kapural et al., 2002; Rapoport, 2000).

Often disruption procedures result in seizures during or within 24 hours of the BBB modification (Roman-Goldstein et al., 1994; Neuwelt et al., 1986; Neuwelt et al., 1983). In fact, seizures are a primary complication of osmotic BBB disruption (Neuwelt et al., 1983) and occur in a relatively large number of patients (13–55%) in spite of AED pretreatment (Roman-Goldstein et al., 1994; Neuwelt et al., 1986; Neuwelt et al., 1983). Neuwelt et al. became increasingly aware of focal-motor and grand mal seizures but attributed them to the use of meglumine iothalamate, a known epileptogenic agent used as a contrast agent for CT. Although seizures continued to occur when BBB disruption was monitored by radionuclide scanning rather than CT, the frequency of occurrence was significantly reduced (Neuwelt et al., 1983). The long-term effects of BBB disruption are unknown, but considering recent findings in animals (see below), these may include hyperexcitability and the formation of an epileptogenic focus. As yet, there is no data available concerning if and to what extent BBB disruption precedes the development of acquired epilepsy in humans.

In addition to iatrogenic BBB disruption, other medical procedures or conditions implicating BBB failure and linked to seizure disorders exist. For example, between 6 and 36% of transplant patients experience seizures, commonly caused by drugs, metabolic derangements or hypoxic-ischemic injury (Patchell, 1994; Gilmore, 1988). Although the seizures are usually transient and easily treated, it has been hypothesized that the focal loss of BBB induced by immunosuppressants may play a significant role in the development of partial seizures in a subpopulation of transplant recipients (Vaughn et al., 1996).

Animal studies

BBB openings have been mapped for a variety of convulsive agents with different mechanisms of action (i.e., impairment of GABA transmission, increased glutamate neurotransmission, direct excitatory action) (Ilbay et al., 2003; Ates et al., 1999; Pont et al., 1995; Suzuki et al., 1984; Nitsch and Klatzo, 1983). Further, with increased arterial blood pressure, the BBB becomes permeable to macromolecules under induced epileptiform seizures (Oztas and Turkel, 2001; Suzuki et al., 1984; Nitsch and Klatzo, 1983). A direct link between the mechanism of action and the region where BBB breakdown was observed is not obvious. However, a few brain regions are easily affected by seizure activity irrespective of the means of induction. Other regions of the brain show BBB breakdown only under the influence of specific convulsants (Nitsch and Klatzo, 1983).

Seizures sometimes appear to be a direct consequence of barrier disruption (see above and (Seiffert et al., 2004; Zappulla et al., 1985a, 1985b; Fieschi et al., 1980)). Zappulla et al. showed that BBB disruption with dehydrocholate results in a slowing of EEG activity and the development of seizure activity, and also that the frequency of seizures increased with further disruptions (Zappulla et al., 1985a). These experiments provided insight into the question of BBB permeability as a component of the etiology of epilepsy or a consequence of seizures. The presence of seizure activity in the contralateral hemisphere in the absence of Evans blue staining in some animals argues against seizure activity as the cause for the increase in BBB permeability observed with dehydrocholate. Based on these findings seizures seem to follow rather than cause disruption induced by bile salts.

In agreement with this, Seiffert et al. attribute the seizures to the exposure of the brain to serum components resulting from the increased permeability of the BBB (Seiffert et al., 2004). They provide evidence that the prolonged BBB disruption induces delayed cortical dysfunction characterized by epileptiform paroxysmal hypersynchronous activity, which persists after the BBB has returned to normal. In addition they demonstrated that the epileptiform activity depends on cortical exposure to low levels of serum albumin. Other serum proteins directly injected into the hippocampus induce both behavioral and electrographic seizures (Xiong et al., 2003).

As the GLUT1 deficiency syndrome revealed, seizures can be related to the local cerebral glucose utilization. Probably not coincidentally, osmotic opening of the BBB has also been tied to abnormalities in local cerebral glucose utilization. Pappius et al. showed that following osmotic opening of the BBB there is a reversible increase in the local cerebral glucose utilization (Pappius et al., 1979). In addition they postulated that because the changes in cerebral energy metabolism parallel changes in levels of local functional activity, the intense neuronal discharges resulting from the BBB disruption can be attributed to several substances normally excluded from brain extracellular spaces. Also, the efficacy of diazepam at inhibiting the glucose utilization in most experiments suggest that some form of seizure activity is responsible for the observed metabolic abnormalities (Pappius et al., 1979).

The results by Seiffert et al. suggest that neurons tend to fire abnormally when exposed to molecules that
extravasate through a leaky BBB (Seiffert et al., 2004). In similar experiments, we studied the abnormal vasculature of rats exposed prenatally to the angiogenic inhibitor thalidomide (Hallene et al., 2005; Krizanac-Bengez et al., 2004b). In humans, the annual incidence of epilepsy in the first 7 years of life is five times that of the general population, and the prevalence of active epilepsy is significantly increased in the teenage thalidomide population. That this increased incidence and prevalence of epilepsy is not a chance observation is supported by published clinical and experimental evidence of central nervous system abnormalities in thalidomide embryopathy, in addition to the known neurological effects of the drug in the adult (Kanno, 1987; Stephenson, 1976).

Abnormal neuronal development in thalidomide rats was associated with vascular malformations and a compromised BBB. Similar findings were reported for MAM-treated rats (Marchi et al., 2005). A brief exposure to intravascular FITC-albumin allowed for exploration of the integrity of the BBB and resulted in a significant accumulation of albumin in neuronal (but, surprisingly, not glial) cells. Thus, it appears that the leakage of the BBB observed in these animals was sufficient to cause extravasation of serum albumin to levels that allowed significant intraneuronal accumulation. Neuronal hyperexcitability was commonly associated with regions of abnormal cortical development typified by protein extravasation. It is therefore possible that the altered neuronal properties of thalidomide and MAM rats are due to a combined “circuitry effect” (e.g., abnormal wiring of neurons) and a concomitant effect of molecules that are normally segregated into peripheral blood.

In conclusion, evidence exists that leakage of the BBB may result in the development of seizures, but a clear cut relationship and the exact nature of the offending mechanisms have remained elusive. This is likely due to the complexity of disease conditions associated with BBB leaks. These include concomitant hemodynamic disturbances (intracerebral hemorrhage or embolic stroke), loss of autoregulation of cerebral blood flow (e.g., in traumatic brain injury), changes in intracranial pressure due to edema, inflammation, etc. Furthermore, the lack of EEG data may actually underestimate the true impact of BBB failure on break down of neuronal control.

**INFLAMMATION, EPILEPSY & BBB**

Traditionally, neuroinflammation has been seen as a CNS-specific branch of immunology. Thus, a great deal of effort has been made to find immunocompetent or inflammatory cells in the brain (or spinal cord) parenchyma. A schematic representation of the cells and molecules involved in cerebral inflammation is shown in Fig. 3. It is now clear that virtually every class of brain cells has some potential or propensity to replicate immunological or inflammatory processes. This emerging field was recently reviewed elsewhere (Vezzani, 2005).

Not all the blood vessels in the brain constitute a BBB: only capillary vessels are endowed with a full-blown BBB phenotype. Vessels of increasing diameter have comparably increasing levels of leakiness and thus superficial vessels of large diameter are the leakiest, while penetrating pial vessels and descending penetrating vessels tend to have an intermediate barrier function. Since most animals, including vertebrates, have some form of barrier separating their blood circulation from the brain or the central nervous system, it has been speculated that profound evolutionary pressure existed to create such a complex organ (Abbott and Pichon, 1987). The central nervous system of vertebrates lacks lymphatic drainage; thus, passage of molecules or ions across the capillary wall will result in a net gain of water into the brain compartment, soon leading to an increase in intracranial pressure. This is a most damaging situation since the brain is contained within a rigid skull. Thus, the combination of a restricted volume and the lack of effective drainage for solutes leaving the blood for the brain parenchyma is probably one of the leading implications for the necessity of a tight barrier between the blood and the brain. The relationship between various BBB compartments (Virchow-Robin space; venules; penetrating pial vessels) has been reviewed in detail elsewhere (Cucullo et al., 2005; Grant and Janigro, 2004).

Our understanding of the cellular mechanisms that initiate changes in BBB permeability is limited. Several vasoactive or inflammatory compounds, which include bradykinin, complement 3α, ATP, histamine and serotonin from mast cells, interleukins, arachidonic acid and its metabolites, interferon alpha and beta, prostaglandins, and tumor necrosis factor, have all been shown to alter BBB permeability (Cucullo et al., 2005; Sinclair et al., 2000a,b; Bartus, 1999; Anderson et al., 1999; Hogue and Ling, 1999; Cass et al., 1998; Faaland et al., 1998; Griffiths et al., 1997; Yao et al., 1997; Diener et al., 1996; Griffith and Jarvis, 1996; Paul, 1995; Washington and Giacomini, 1995; Davies et al., 1977). A subsequent rise in intracellular calcium may stimulate cyclic nucleotide production, which in turn leads to pinocytosis and vesicular transport. It has been proposed that the rise in intracellular calcium also triggers a contraction of the endothelial cells, which increases permeability by deforming or opening the intercellular tight junctions. The role of vasoactive agents in the control of BBB permeability, edema formation, and leukocyte infiltration is a key field of study. Given the prominent role of BBB integrity in the control of brain homeostasis and neuronal excitability, it is simple to predict that inflammatory changes affecting BBB integrity may have a profound impact on brain function.

The tightness of the BBB is a serious hindrance to the entry of both immunocompetent cells and specific antibodies, which are necessary if the immune system is to
FIG. 3. Summary of events that may link intravascular inflammatory events to pro-epileptogenic events in the brain parenchyma. Please note that this schematic representation does not implicate the presence of a particular pathogen, and may actually occur under sterile inflammatory conditions(Krizanac-Bengez et al., 2006; Krizanac-Bengez et al., 2004a; Krizanac-Bengez et al., 2003). Under normal conditions, an intact BBB separates the immune system from the CNS parenchyma. Trafficking of white blood cells is restricted to specific regions of the vasculature, namely the Virchow-Robin space above the leptomeningeal fusion, and the subarachnoid space(Grant and Janigro, 2004). When the BBB is breached, both molecular (e.g., complement) and cellular players may extravasate. The mechanism of this BBB attack by intravascular agents implicates metalloproteinases and other molecules released by activated blood cells. The abnormal permeation across the barrier results in further, and perhaps distal, disruption of tight junctions, this time mediated by release of inflammatory mediators by both extravasated blood cells and activated microglia. Frank cellular immunoagression occurs if and when histocompatibility mechanisms are activated and antibody-mediated reactions occur. IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; CSF, colony-stimulating factor; MHC, major histocompatibility complex.

attack infectious agents or abnormal autologous cells undergoing uncontrolled proliferation in the brain. The highly specialized tight endothelium isolates the brain from immune surveillance and allows only a few mononuclear cells (activated T cells) to migrate into the CNS (Silverman et al., 2000). Therefore, the low expression of major histocompatibility complex (MHC) antigens, the low number of antigen-presenting cells, and the fact that the CNS is not drained by a fully developed lymphatic vasculature, makes the brain an “immunoprivileged” site. However, when inflammation does occur, there is extensive leukocyte migration into the brain. Both brain endothelial cells and astrocytes can act as antigen presenting cells in order to facilitate the entry of T-lymphocytes.
and antibodies. The BBB itself plays an active role in the mediation of this neuroimmune response, either by the production of inflammatory mediators or by expression of adhesion molecules.

Cytokines are cellular hormones such as neurotrophins, neuropaoptiogenic factors, interleukins (IL-1 and IL-6), interferons, colony stimulating factors, growth factors, and thymic hormones that are released following stress. Cells in the CNS that can produce cytokines upon activation include macrophages, microglial cells, astrocytes, and cerebral endothelial cells (de-Boer and Breimer, 1998). Cytokines may influence the transport of compounds into the brain by opening the BBB. Studies revealed that the administration of IL-1, IL-6, TNF-α, and IFN-γ, increase in endothelial permeability (Schilling and Wahl, 1999; Laflamme et al., 1999; Freyer et al., 1999; Rivest, 1999). TNF-α has been shown to induce the production of matrix metalloproteinases, in particular gelatinase B, which in turn attacks the basal lamina macromolecules like Type IV collagen surrounding brain microvessels. Gelatinase A is associated with tumor invasion and angiogenesis. Derivatives of arachidonic acid, called eicosanoids, also play an important role in the mediation of the inflammatory response. Arachidonic acid is released from cell membrane phospholipids during inflammation by phospholipase A2 (inhibited by corticosteroids). Oxidative metabolism of arachidonic acid produces prostaglandins and thromboxane A2 via the cyclooxygenase and lipooxygenase pathways. All of these agents have been shown to modulate BBB function. Within minutes after the release of such inflammatory mediators, neutrophils arrive at the site of inflammation followed by antigen specific B and T lymphocytes and monocytes into the inflamed site (immunoglobulin (Ig) super family, integrins, and selectins). Brain endothelial cells are capable of expressing several adhesion molecules including, intercellular adhesion molecule-1 (ICAM-1), ICAM-2, and vascular cell adhesion molecule-1 (VCAM-1). The promotion of leukocyte adhesion to the cerebral endothelium may be mediated through a modulation of these adhesion molecules which then permits the migration of lymphocytes across the BBB via a transcellular route. The expression of major MHC-I and MHC-II is also controlled by cytokines.

The first cell type recognized with acknowledged immunological and inflammatory potential was microglia (Benveniste, 1992). Most current evidence strongly suggests that microglia are derived from a bone marrow precursor of monocytic lineage and populate the CNS early in fetal development. In addition, there is immigration during the postnatal period. Thus, while microglia resides in the CNS, it does not derive from CNS precursors. Microglia have functions similar to those of other tissue macrophages, including phagocytosis, antigen presentation, and production of cytokines, eicosanoids, complement components, excitatory amino acids (glutamate), proteinases, oxidative radicals, and nitric oxide (Thomas, 1992).

In addition to intrinsic inflammatory mechanism, it is increasingly clear that the peripheral immune system may, under certain circumstances, provoke havoc in the CNS. This is a rare occurrence, however, thanks in part to a BBB mechanism that (1) impedes or hampers cell migration across the endothelial cell monolayer; (2) prevents or reduces chemotraction of potentially harmful macrophages. The flipside of this is that a CNS-specific antigen may be considered as nonself and thus lead to autoimmunity. Again, even when this happens the BBB minimizes the risks associated with the presence of offending effector cells in the peripheral circulation.

**Animal studies**

The evidence of inflammatory processes in the clinical manifestations and neuropathological sequelae of epilepsy have accumulated in the last decade (Vezzani, 2005; Vezzani, 2004; Vezzani et al., 2002). For example, administration of kainic acid, an analogue of the excitatory amino acid glutamate, induces a characteristic behavioral syndrome and a reproducible pattern of neurodegeneration in several brain areas, closely resembling human temporal lobe epilepsy (Oprica et al., 2003). It has been shown that manipulation of pro- and antiinflammatory cytokines can modify the outcome, as well as the neuropathological consequences, of experimentally induced seizures (Vezzani et al., 2002). For example, IL1-β is involved in the initiation of early stages of inflammation and plays a pivotal role in the neuroinflammation associated with certain forms of neurodegeneration, including cerebral ischemia, trauma and excitotoxic brain injury. Several studies have suggested that at least in the case of febrile seizures IL1-β can facilitate the development of seizures (Dube et al., 2005).

There are several potential explanations for the conflicting results obtained with animal models. One of the issues that has not been fully explored is whether the increased or decreased levels of cytokines were a consequence of or prodromic to the seizures. In addition to the temporal sequence of cause/effects, the cellular origin is also important. It is commonly assumed that “brain” cytokines derive in fact from brain cells, while quantitatively white blood cells are the main source of interleukins. In fact, the release of inflammatory mediators by blood cells results in focal BBB failure and this may facilitate extravasation of substantial quantities of cytokines. This is likely followed by binding to CNS receptors with a plethora of downstream effects. Fig. 3 summarizes some of these events. An additional confounding factor is the type, duration and intensity of seizures. All these features combined may well produce opposite effects on activation of cells either present in the brain or transmurally influenced by a
number of hemodynamic changes that are associated with seizures.

In conclusion, a number of variables have to be considered and experimentally controlled before the exact nature and consequence of inflammation in animal models of seizures can be understood. In any case, as in the case of more obvious neurological disorders based on immune overreaction (e.g., MS), the BBB status is likely to play an important role.

Human studies
Clinical evidences link inflammation of the CNS to the development of seizures. In Rasmussen’s syndrome, a very rare form of brain malfunction which may occur at any time in childhood, it is known that brain cells usually in only one hemisphere are inflamed. Rasmussen’s encephalitis was originally thought to be a chronic form of viral encephalitis but is now considered to be an autoimmune disease of the brain and is more properly termed Rasmussen’s syndrome. Starting in one area of one side of the brain, the disease appears to gradually and progressively involve that side of the brain causing progressive and intractable focal seizures, a hemiparesis, and expressive aphasia when the left hemisphere is involved. Immune therapy with steroids, immunoglobulins, or plasmapheresis provides only temporary relief from seizures.

How Rasmussen’s relates to other epilepsies in terms of etiology and pathology, relationship to seizure focus, and origin of offending cells and mediators has yet to be fully elucidated. However, it is remarkable that the animal studies led to the hypothesis that brain-derived inflammatory mediators and cells are “activated” or released, while it is clear that in Rasmussen’s the origin is systemic. It is thus possible that seizures influence the immune system of humans in a fashion that is not replicable in animal models. An excellent recent review by Vezzani and Granata addressed most of the issues linking inflammation to the BBB and epilepsy (Vezzani and Granata, 2005).

CONCLUSION
The BBB is intimately interconnected with the cause, effect and treatment of seizures. These relationships continue to move toward the forefront of epilepsy research and offer a distinctive opportunity to further our understanding of the disease. With the constant development of new technologies and refinement of existing technologies, our ability to image, manipulate and explore the BBB will only improve, thereby enabling the next generation of advances.

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