

DOES LEAKAGE OF THE BLOOD–BRAIN BARRIER MEDIATE EPILEPTOGENESIS?

Blood-Brain Barrier Leakage May Lead to Progression of Temporal Lobe Epilepsy. van Vliet EA, da Costa Araujo S, Redeker S, van Schaik R, Aronica E, Gorter JA. *Brain* 2007;130(Pt 2):521–534. Leakage of the blood–brain barrier (BBB) is associated with various neurological disorders, including temporal lobe epilepsy (TLE). However, it is not known whether alterations of the BBB occur during epileptogenesis and whether this can affect progression of epilepsy. We used both human and rat epileptic brain tissue and determined BBB permeability using various tracers and albumin immunocytochemistry. In addition, we studied the possible consequences of BBB opening in the rat for the subsequent progression of TLE. Albumin extravasation in human was prominent after status epilepticus (SE) in astrocytes and neurons, and also in hippocampus of TLE patients. Similarly, albumin and tracers were found in microglia, astrocytes and neurons of the rat. The BBB was permeable in rat limbic brain regions shortly after SE, but also in the latent and chronic epileptic phase. BBB permeability was positively correlated to seizure frequency in chronic epileptic rats. Artificial opening of the BBB by mannitol in the chronic epileptic phase induced a persistent increase in the number of seizures in the majority of rats. These findings indicate that BBB leakage occurs during epileptogenesis and the chronic epileptic phase and suggest that this can contribute to the progression of epilepsy.

TGF-Beta Receptor-Mediated Albumin Uptake into Astrocytes Is Involved in Neocortical Epileptogenesis. Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tomkins O, Seiffert E, Heinemann U, Friedman A. *Brain* 2007; 130(Pt 2):535–547. It has long been recognized that insults to the cerebral cortex, such as trauma, ischaemia or infections, may result in the development of epilepsy, one of the most common neurological disorders. Human and animal studies have suggested that perturbations in neurovascular integrity and breakdown of the blood–brain barrier (BBB) lead to neuronal hypersynchronization and epileptiform activity, but the mechanisms underlying these processes are not known. In this study, we reveal a novel mechanism for epileptogenesis in the injured brain. We used focal neocortical, long-lasting BBB disruption or direct exposure to serum albumin in rats (51 and 13 animals, respectively, and 26 controls) as well as albumin exposure in brain slices *in vitro*. Most treated slices (72%, $n = 189$) displayed hypersynchronous propagating epileptiform field potentials when examined 5–49 days after treatment, but only 14% ($n = 71$) of control slices showed similar responses. We demonstrate that direct brain exposure to serum albumin is associated with albumin uptake into astrocytes, which is mediated by transforming growth factor β receptors (TGF- β R). This uptake is followed by down regulation of inward-rectifying potassium (Kir 4.1) channels in astrocytes, resulting in reduced buffering of extracellular potassium. This, in turn, leads to activity-dependent increased accumulation of extracellular potassium, resulting in facilitated *N*-methyl-D-aspartate-receptor-mediated neuronal hyperexcitability and eventually epileptiform activity. Blocking TGF- β R *in vivo* reduces the likelihood of epileptogenesis in albumin-exposed brains to 29.3% ($n = 41$ slices, $P < 0.05$). We propose that the above-described cascade of events following common brain insults leads to brain dysfunction and eventually epilepsy and suggest TGF- β R as a possible therapeutic target.

COMMENTARY

A flurry of recent papers confirms the growing interest in cerebrovascular research among epileptologists (1). After the early pioneering work by Quadbeck and Helmchen, who suggested that loss of blood–brain barrier (BBB) integrity may lead to a variety of CNS disorders including seizures, almost half a century has elapsed without significant advances in research on the BBB as it relates to epilepsy (2). In fact, most of the work on the BBB and epilepsy has focused on multiple drug resistance, with little acknowledgment of an etiologic role for cerebrovascular failure in seizure disorders. It is now known, at least in principle, that BBB disruption leads to acute seizures in humans and animal models (3,4). The two papers reviewed here

further investigate the mechanisms (Ivens et al.) and etiology (van Vliet et al.) of BBB disruption in seizure disorders.

The work by Ivens and colleagues is a logical continuation of earlier studies that induced seizures in rats by exposing the brain surface to bile salt, which is believed to “open” the BBB (4). One of the most significant findings using this model is a persistent and dramatic ingress of extravasated serum albumin into astrocytes. The finding and its relationship to abnormal electrical activity were further investigated, and it was demonstrated that albumin loading of CNS glia is mediated by a specific receptor for TGF- β , a powerful regulator of apoptosis and the cell cycle. Interestingly, the putative downstream event of this altered signaling is one of the oldest suspects in epileptogenesis, namely, increases in extracellular K^+ . Furthermore, the current hypothesis links cell cycle, gliosis, and expression of potassium channels, as was anticipated by Dini et al. (5).

The paper by van Vliet and colleagues tackles another aspect of the link between vascular and parenchymal factors in

epileptogenesis. The authors showed that in the kainate model of epilepsy there is impairment of the BBB and that loss of cerebrovascular protection may be one factor in determining epileptogenesis. Thus, treatment with an osmotic agent commonly used to treat brain edema, leads to BBB leakage that is associated with an increased probability of ictal activity. The study employed standard intravascular staining techniques to demonstrate BBB leakage. Clinically, BBB integrity is assessed with gadolinium-enhanced MRI. In the laboratory, BBB function is commonly determined with markers that bind serum albumin or albumin itself conjugated to fluorophores, such as fluorescein isothiocyanate, or to Evans blue. Using this technique, Seiffert et al. described albumin accumulation into astrocytes. In the study by van Vliet, however, albumin accumulated equally well in neurons, confirming results by others (3,6).

van Vliet et al. did not explore the mechanism by which BBB leakage may contribute to epileptogenesis. Nevertheless, whatever the mechanisms, BBB failure triumphantly enters the crowded field of epileptogenic triggers. There are reasons for both cheers and jeers. The involvement of the BBB in epilepsy opens new therapeutic options, particularly when and if the targets are known and accessible. For example, assuming TGF- β is relevant, as proposed by Seiffert et al., it might be worthwhile to attempt to modulate the expression of this protein by antisense or small interfering RNA (siRNA) technology. Loss of BBB function commonly results from inflammatory changes associated or not associated with trauma, suggesting a link between seizures, the BBB, and inflammation (1). In addition to acute seizures, there is new evidence that inflammation may also play a role in epileptogenesis, although no antiinflammatory compounds have yet been shown to be protective (7). Chronic immunosuppression is fraught with concerns. However, short-term immunosuppression during the period of vulnerability following an epileptogenic stimulus, might find utility. Several issues and incongruities need to be resolved before a rational, BBB-based therapeutic approach is ready for clinical application. These include using comparable means to study the BBB in human subjects and animal models.

First and foremost, in the study of van Vliet et al., the treatment used to open the BBB is administered clinically to protect against seizures. In fact, at the concentrations these authors used, intravenous mannitol slightly elevates blood osmolarity and is commonly employed to decrease intracranial pressure via a simultaneous osmotic action on the kidney and the brain. At significantly higher concentrations (1.4 molar) and when applied intraarterially to the carotid or vertebral circulation, mannitol is used to open the BBB. When the latter procedure was used, acute seizures resulted (3). It is unclear at what concentration or dose the effect of mannitol changes from protective to damaging, and the mechanisms underlying this shift are still unknown.

The link between loss of BBB function and albumin accumulation in glia also needs further investigations. The hypothesis formulated regarding the specificity of albumin accumulation in astrocytes is not necessarily at odds with the fact that van Vliet et al. and others (3,6) found albumin in neurons as well. In fact, the data convincingly show that a small decrease in spatial buffering of extracellular K^+ occurred after exposure to albumin. However, the alternative hypothesis implicating an effect of albumin on potassium currents also should be considered. A direct action of albumin acting on potassium channels is made even more intriguing by the fact that the very method Ivens et al. used to induce epileptogenesis—bile salts—also inhibits potassium channel activity (8).

Ivens et al. found that a specific inwardly rectifying current was reduced by albumin, namely the inwardly rectifying potassium 4.1 (Kir 4.1) channel. Kir, and in particular Kir 4.1, are key regulators of glial functions, which in turn determine neuronal excitability and axonal conduction (9,10). The electrophysiological characterization of astrocytes from Kir 4.1 knockout mice showed that Kir 4.1 mediates most of the Kir current in astrocytes, but the fact that loss of Kir 4.1 did not significantly alter neuronal function suggests that these channels are one player among many in the coordinated process of extracellular potassium regulation. In the paper by Ivens et al., the effect of albumin on extracellular K^+ also was modest, suggesting that even in this model, alternative mechanisms to buffer extracellular potassium are present or induced.

In summary, these two studies further an understanding of how and why BBB opening leads to seizures and epileptogenesis. There now is overwhelming evidence that these mechanisms may have an important etiological role in acute or iatrogenic human seizures as well as in animal models. There are still several aspects to be elucidated, and consensus must be reached on how clinically relevant procedures (e.g., BBB disruption to treat brain tumors) and experimental approaches (e.g., bile salts, low concentrations of mannitol) can be reconciled. Perhaps, the most surprising findings of the study by van Vliet is the fact that epileptogenesis was induced by procedures that are clinically used to prevent seizures and neuronal damage. In any event, both studies demonstrate the urgent need for new strategies to improve BBB function or to prevent its breakdown during seizures.

by Damir Janigro, PhD

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