

## A POSSIBLE CAUSATIVE ROLE FOR BLOOD–BRAIN BARRIER FAILURE AND REACTIVE ASTROCYTOSIS IN ACQUIRED EPILEPSY

### Lasting Blood–Brain Barrier Disruption Induces Epileptic Focus in the Rat Somatosensory Cortex

Seiffert E, Dreier JP, Ivens S, Bechmann I, Tomkins O, Heinemann U, Friedman A

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Perturbations in the integrity of the blood–brain barrier have been reported in both humans and animals under numerous pathologic conditions. Although the blood–brain barrier prevents the penetration of many blood constituents into the brain extracellular space, the effect of such perturbations on the brain function and their roles in the pathogenesis of cortical diseases are unknown.

In this study, we established a model for focal disruption of the blood–brain barrier in the rat cortex by direct application of bile salts. Exposure of the cerebral cortex *in vivo* to bile salts resulted in long-lasting extravasation of serum albumin to the brain extracellular space and was associated with a prominent activation of astrocytes with no inflammatory response or marked cell loss. By using electrophysiological recordings in brain slices, we found that a focus of epileptiform discharges developed within 4 to 7 days after

treatment and could be recorded up to 49 days postoperatively in more than 60% of slices from treated animals but only rarely (10%) in sham-operated controls. Epileptiform activity involved both glutamatergic and GABAergic neurotransmission. Epileptiform activity also was induced by direct cortical application of native serum, denatured serum, or albumin-containing solution. In contrast, perfusion with serum-adapted electrolyte solution did not induce abnormal activity, thereby suggesting that the exposure of the serum-devoid brain environment to serum proteins underlies epileptogenesis in the blood–brain barrier–disrupted cortex. Although many neuropathologies entail a compromised blood–brain barrier, this is the first direct evidence that it may have a role in the pathogenesis of focal cortical epilepsy, a common neurologic disease.

### COMMENTARY

The blood–brain barrier (BBB) is a structure designed to maintain a controlled neuronal environment by limiting the exchange of a wide range of molecules between the blood and the brain. It is composed of endothelial cells interconnected by tight junctions, a functional specialization that is controlled by surrounding astrocytic endfeet. Virtually all insults to the CNS, such as trauma, stroke, brain tumors, and epileptic seizures, are associated with a pathologically increased permeability of the BBB.

In the specific case of epilepsy acquired after CNS insult, the mechanisms of its genesis and progression remain poorly understood. However, it has long been hypothesized that the pathologic breakdown of the BBB may contribute to epileptogenesis by allowing the penetration of proepileptic blood-borne molecules into the brain. Induction of robust, recurrent, and spontaneous seizures after focal application of ferric or ferrous ions (present in whole blood) into rat and cat cortices provide

evidence that this may be the case as well as evidence for the hypothesis that human posttraumatic epilepsy may result from blood extravasation and subsequent iron-mediated peroxidation of brain cell membranes (1,2). However, the demonstration that, after epileptogenic head injury in humans, iron salts are indeed deposited intraparenchymally in sufficient quantity to generate an epileptic focus has been lacking. In addition, it is becoming increasingly clear that other blood-borne molecules are neurotoxic and proinflammatory when penetrating into the brain and, therefore, have the potential, alone or in synergy, to be proepileptic after a pathologic breakdown of the BBB. An example is thrombin, which was found to induce microglial activation and production of reactive oxygen species (3) and to be sufficient to induce seizures (4).

The recent study by Seiffert and coworkers, by using an elegant experimental approach, significantly contributes to this investigative line by shedding light on the role of focal BBB opening and serum/albumin extravasation in the development of chronic cortical hyperexcitability. The authors first induced *in vivo* focal BBB disruption of the rat somatosensory cortex by subdural perfusion (less than 1 hour) of the exposed cortex with artificial CSF containing bile salts. Significant BBB opening was demonstrated to persist at least 6 days after treatment.

To exclude that application of bile salts could be directly neurotoxic, neocortical slices were studied *in vitro* during similar bile salt application, and pathological analysis was performed. Both intracellular and extracellular electrophysiologic recordings revealed no changes in evoked neuronal activity *in vitro*. Pathological analysis did not reveal gross neuronal loss. However, the long-term effects of *in vivo* BBB disruption were evident in the persistent focal neuronal hyperexcitability observed by *in vitro* recordings from slices obtained 2 hours to 49 days after treatment. This *in vitro* hyperexcitability was specifically associated with the treatment site and was never observed in untreated areas of the same slices or in slices from sham-operated rats.

To rule out further the possibility that it was a specific neurotoxic effect of bile salts that induced chronic neocortical hyperexcitability and to probe the mechanisms of generation of neocortical hyperexcitability, the authors performed *in vivo* focal perfusion of the rat neocortex with rat serum, which also was found to induce chronic neocortical hyperexcitability. Similar results were obtained with focal application of weakly denatured serum (incubated at 70°C for 1 hour) and with artificial CSF supplemented with albumin from 100% to 25% of normal serum albumin levels, therefore demonstrating the relevance of the finding.

*In vitro* experiments with control neocortical slices superfused with albumin for up to 2 hours did not produce a proepileptic effect, suggesting that the proepileptic changes induced by focal BBB opening may be due to slow processes triggered *in vivo* by the increased extracellular levels of serum proteins. Inflammation-related processes appear to be good candidates to explain this phenomenon; however, histologic staining did not detect penetration of macrophages or red cells or gross neuronal loss after focal application of albumin or bile salts. In addition, no increase in inflammation was observed by immunostaining with antibodies against CD40 and GSA-isolectin B4. However, a prominent increase in immunostaining of glial fibrillary acidic protein (GFAP), a cytoskeletal marker of glial reactivity, was observed 2 to 14 days after treatment, suggestive of a rapid and persistent astrocytosis in both bile salt-treated and albumin-treated cortices.

The experiments by Seiffert and coworkers demonstrate that serum extravasation is, *per se*, proepileptic; further work will be needed to elucidate whether it also is epileptogenic, that is, sufficient to generate chronic spontaneous recurrent seizures. If serum extravasation is not found to be epileptogenic, it will be useful to determine how potent a proepileptic agent it is relative to other agents (e.g., iron and thrombin) that act after CNS insult. The data suggest strongly, but not conclusively, that albumin is a major proepileptic factor in the serum. The possibility exists that the observed proepileptic action of albumin may actually be due to a biologic contaminant. Indeed, large-scale manufacturers typically obtain albumin at only 96%

to 99% purity. Possible proepileptic contaminants of albumin could include fragments of hemoglobin, thrombin, transferrin, and other substances at concentrations sufficient to be immunogenic or neuroactive.

The beauty of the work of Seiffert and coworkers is that it demonstrates that the predominant pathologic feature of the neocortex, after a spatially limited and carefully controlled BBB opening, is a pronounced reactive astrocytosis (as assessed by GFAP immunostaining), which first precedes and then accompanies the chronic neuronal hyperexcitability, suggesting its possible causative role in the observed chronic hyperexcitability. What are the mechanisms by which albumin and serum protein extravasation could result in reactive astrocytosis and in chronic neocortical hyperexcitability? The finding that neocortex also became hyperexcitable after focal application of denatured serum argues against a specific ligand-receptor interaction, although this cannot be fully ruled out now because weakly denatured protein may maintain bioactivity. Indeed, it is known that microglia can be activated by denatured protein. Furthermore, enzymatic activities or bioactive molecules contaminating albumin may be resistant to heat-based denaturing protocols. It is intriguing that albumin very recently was found to activate microglia promoting proliferation and intracellular calcium oscillation (5). This discovery, therefore, could explain a possible link between albumin extravasation and reactive astrocytosis. However, Seiffert and coworkers did not observe a significant increase in CD40 and GSA-isolectin B4—inflammatory markers expressed in the cell membranes of activated microglia. One is left wondering whether albumin could possibly induce microglia proliferation without resulting in CD40 and IB4 overexpression. Another possibility, not discussed by the authors, is that albumin and serum may be proepileptic by binding and sequestering endogenous antiepileptic or antiinflammatory factors or both, ranging from antiinflammatory-antiepileptic cytokines or inhibitors of proepileptic cytokine receptors (6,7) to endogenous modulators of GABAergic or glutamatergic synaptic transmission. Further work will be needed to investigate this hypothesis and the mechanisms of serum-induced epileptogenesis.

Once the focal BBB opening results in a focus of glial/astrocytic reactivity, what might be the cascade of mechanisms that result in chronic neuronal hyperexcitability? It is becoming increasingly clear that certain reactive astrocytes may undergo changes that are proepileptic (7,8). For example, post-traumatic reactive glia have a reduced activity of membrane K<sup>+</sup> channels that are known to be necessary for proper buffering of extracellular K<sup>+</sup>, and as a result, potassium builds up in the extracellular space, lowering seizure threshold (9,10). Astrocytes also have a major role in recycling the neurotransmitter glutamate, handling about 90% of all glutamate clearance. However, posttraumatic reactive astrocytes have a decreased expression of

the glutamate transporter protein GLT-1 at chronic time points after controlled cortical impact (11) and in the ferrous chloride model of posttraumatic epilepsy (12). More evidence has been accumulating that when glial cells are genetically engineered to overproduce proinflammatory cytokines in mice, the animals become more prone to drug-induced seizures. Yet, when astrocytes are genetically engineered to lack the receptor for one of these cytokines, interleukin (IL)-1 $\beta$ , mice are less prone to seizures, as are mice treated with a drug that blocks the IL-1 $\beta$  receptor (6,7).

In summary, evidence has been accumulating that reactive astrocytosis is proepileptic. The discovery that focal opening of the BBB induced early focal glial reactivity and late chronic neuronal hyperexcitability invites further investigation into the mechanisms of blood-induced reactive astrocytosis and glial mechanisms of acquired epileptogenesis.

by Raimondo D'Ambrosio, PhD

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