NOT AGAIN! THE ROLE OF BLOOD–BRAIN BARRIER FAILURE IN EPILEPTOGENESIS: A MOLECULAR UPDATE

Transcriptome Profiling Reveals TGF-β Signaling Involvement in Epileptogenesis. Cacheaux LP, Ivens S, David Y, Lakhter AJ, Bar-Klein G, Shapira M, Heinemann U, Friedman A, Kaufer D. *J Neurosci* 2009;29(28):8927–8935. Brain injury may result in the development of epilepsy, one of the most common neurological disorders. We previously demonstrated that albumin is critical in the generation of epilepsy after blood–brain barrier (BBB) compromise. Here, we identify TGF-β pathway activation as the underlying mechanism. We demonstrate that direct activation of the TGF-β pathway by TGF-β1 results in epileptiform activity similar to that after exposure to albumin. Coimmunoprecipitation revealed binding of albumin to TGF-β receptor II, and Smad2 phosphorylation confirmed downstream activation of this pathway. Transcriptome profiling demonstrated similar expression patterns after BBB breakdown, albumin, and TGF-β1 exposure, including modulation of genes associated with the TGF-β pathway, early astrocytic activation, inflammation, and reduced inhibitory transmission. Importantly, TGF-β pathway blockers suppressed most albumin-induced transcriptional changes and prevented the generation of epileptiform activity. Our present data identifies the TGF-β pathway as a novel putative epileptogenic signaling cascade and therapeutic target for the prevention of injury-induced epilepsy.

Astrocytic Dysfunction in Epileptogenesis: Consequence of Altered Potassium and Glutamate Homeostasis? David Y, Cacheaux LP, Ivens S, Lapilover E, Heinemann U, Kaufer D, Friedman A. *J Neurosci* 2009;29(34):10588–10599. Focal epilepsy often develops following traumatic, ischemic, or infectious brain injury. While the electrical activity of the epileptic brain is well characterized, the mechanisms underlying epileptogenesis are poorly understood. We have recently shown that in the rat neocortex, long-lasting breakdown of the blood–brain barrier (BBB) or direct exposure of the neocortex to serum-derived albumin leads to rapid upregulation of the astrocytic marker glial fibrillary acidic protein, followed by delayed (within 4–7 day) development of an epileptic focus. We investigated the role of astrocytes in epileptogenesis in the BBB-breakdown and albumin models of epileptogenesis. We found similar, robust changes in astrocytic gene expression in the neocortex within hours following treatment with deoxycholic acid (BBB breakdown) or albumin. These changes predict reduced clearance capacity for both extracellular glutamate and potassium. Electrophysiological recordings *in vitro* confirmed the reduced clearance of activity-dependent accumulation of both potassium and glutamate 24 hour following exposure to albumin. We used a NEURON model to simulate the consequences of reduced astrocytic uptake of potassium and glutamate on EPSPs. The model predicted that the accumulation of glutamate is associated with frequency-dependent (>100 Hz) decreased facilitation of EPSPs, while potassium accumulation leads to frequency-dependent (10–50 Hz) and NMDA-dependent synaptic facilitation. *In vitro* electrophysiological recordings during epileptogenesis confirmed frequency-dependent synaptic facilitation leading to seizure-like activity. Our data indicate a transcription-mediated astrocytic transformation early during epileptogenesis. We suggest that the resulting reduction in the clearance of extracellular potassium underlies frequency-dependent neuronal hyperexcitability and network synchronization.

COMMENTARY

There is little doubt that the blood–brain barrier (BBB) is now a recognized centerpiece of the puzzle of epileptogenesis. After initial descriptive studies, the field has rapidly evolved and two main concepts have emerged: 1) BBB disruption can induce "acute seizures" in rodents and human subjects, and systemic pilocarpine administration in rodents causes status epilepticus by opening the BBB (1–4); and 2) "Delayed epileptogenesis" is mediated by an interplay involving endothelia-astrocyte signaling (perhaps, triggered by BBB disruption) (5). The latter concept is the focus of the papers by Cacheaux et al. and David et al. The immediate development of seizures after acute BBB disruption is likely to depend on rapid (minutes) diffusion of serum contents across a leaky BBB or by rapid (tens of minutes) activation of the immune system, which can be prevented by inhibition of serum interleukin-1 (IL-1) β signaling or leukocyte–endothelial interactions (1,2). The time interval of the experimental epileptogenesis models (several hours in vitro and 4 days in vivo) suggests a transcription-mediated mechanism.

The process described in the papers highlighted here deals with delayed epileptogenesis and shows that in addition to

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peripheral inflammatory markers, new targets have been unveiled. Both studies have elegantly shown that the molecular effector that links BBB failure to astrocytic dysfunction is albumin acting on a specific receptor: transforming growth factor-β (TGF-β). The experimental design is simple yet remarkable for its reproducibility. In the paper by Cacheaux and colleagues, an in vivo model of progressive BBB disruption is obtained by deoxycholic acid applied to the cortex, which results in the gradual development of hypersynchronous neuronal epileptiform activity. The in vitro model, used in the study by David and colleagues, is based on the direct application of albumin on brain slices; the procedure also causes spontaneous and evoked neuronal discharges that are similar to the EEG recordings obtained in vivo. The albumin and deoxycholic acid mechanisms proposed by Cacheaux et al. and David et al., respectively, may both be downstream effectors of early ictal events, such as inflammatory activation of vascular adhesion molecules or increased serum IL1-β (1,2).

There are two significant features of these models: the similarities of outcomes in vivo and in vitro and the interchangeability of triggers (deoxycholic acid or albumin). In fact, both models and both agents implicate the same, specific glial receptor, TGF-β, as mentioned. Once they established these experimental outcomes, the authors used state-of-art cDNA array methods with two goals: 1) to describe the pathways downstream of TGF-β signaling (Cacheaux et al.), and 2) to construct a computer model describing the electrophysiological and functional changes that underlie the hyperexcitable state measured after exposure to BBB disruptors or albumin (David et al.). The results unveil a novel set of genes that mediate TGF-β actions and a mechanism of neuronal hyperexcitability that results from altered glial management of extracellular potassium ($[K^+]_{out}$) and the neurotransmitter, glutamate. The two processes are linked by a common thread: a glial resting membrane potential in which potassium influx is favored by a negative astrocytic membrane potential and glutamate uptake is driven by a sodium gradient.

The evidence that blockade of potassium uptake can promote synchronization and seizure-like activity in brain slices is not novel (6), and the concept of potassium buffering by astrocytes and other glial subtypes is equally well established (1). What, then, is the novelty of these two studies? The original work was performed by using nonphysiological blockers of $[K^+]_{out}$ uptake. In particular, the ion cesium was found not only to depolarize glial cells but also to reduce potassium fluxes into the glial syncytium. The subsequent quest for endogenous inward rectifier potassium-channel (K_{ir}) blockers has revealed only a few viable candidates, albumin being one of the most appealing for the reasons outlined in the manuscripts reviewed here. In addition, the description of a downstream cascade of extracellular events, provided by the authors, is clearly a first.

In Cacheaux et al., the authors report that TGF-β signaling is sufficient to induce epileptiform activity and that it is the mechanism by which albumin causes delayed-onset seizures. In addition, the accompanying paper by David et al. shows that an early and prominent change in astrocytic gene expression is an important early feature of BBB breakdown and albumin-induced epileptogenesis. This finding indirectly but convincingly shows that the endogenous effector of reduced $[K^+]_{out}$ regulation after BBB disruption is indeed albumin acting on TGF-β receptors. By a combination of routine gene chip analysis and historical data from the literature, the authors were able to construct a computer model that recapitulates some of the neuronal changes that one would predict to occur when glial function is affected. Most of the features are consistent with what basic scientists would expect to see in a model of early epileptogenesis. Thus, the model is likely to become a useful tool to study how various interventions, such as BBB disruption, affect synaptic transmission.

There are a number of future developments that may result from this model and, in general, from albumin as a pathological agent responsible for post-BBB disruption epileptogenesis. However, first there is a need for a cross comparison with data sets obtained from other models of epilepsy in which BBB disruption is the event that triggers the initial seizure. For example, both osmotic BBB disruption (4) and pilocarpine (3,7) may act by interrupting normal potassium homeostasis. A cross comparison would add impetus to a synergistic research effort that might link different models of seizures and epileptogenesis to meaningful mechanisms of clinical relevance. It is well established that BBB disruption, as seen after traumatic brain injury, inflammation or fever, and cerebrovascular accidents, is a chief epidemiologic trigger of both acute seizures and delayed epileptogenesis. Opening of the BBB caused by yet unknown inflammatory triggers or by infection, stroke, and transient ischemic attacks might set the process in motion. Such events might cause a first seizure (perhaps directly triggered by extravasating potassium ions, as suggested by David et al. and Janigro et al. (6) among others) that is accompanied by albumin leakage. BBB leakage has been demonstrated in rodent models (2) as well as in human tissue (8). After the initial event, the cascade described in these papers will transform an initial ictal event into an epileptogenic process that may results in spontaneous seizures.

There are two considerations that may slightly dampen enthusiasm for these papers. First, several investigators have seen abundant albumin uptake by neurons and not by glia (9). The relevance of this seemingly conflicting finding, given that different experimental approaches were used, is unclear. In addition, it has not yet been shown that TGF-β receptors in human brain are located in proximity to regions of BBB leakage. If this turns out to be the case, as is expected given the results in rodents,

it will become important to understand if this mechanism is unique to epileptic brain or a feature common across the broad spectrum of neurological diseases that are characterized by a leaky BBB (10).

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