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Leukocyte–Endothelial Adhesion Mechanisms in Epilepsy: Cheers and Jeers

A Role for Leukocyte-Endothelial Adhesion Mechanisms in Epilepsy. Fabene PF, Navarro MG, Martinello M, Rossi B, Merigo F, Ottoboni L, Bach S, Angiari S, Benati D, Chakir A, Zanetti L, Schio F, Osculati A, Marzola P, Nicolato E, Homeister JW, Xia L, Lowe JB, McEver RP, Osculati F, Sbarbati A, Butcher EC, Constantin G. *Nat Med* 2008;14(12):1377–1383. The mechanisms involved in the pathogenesis of epilepsy, a chronic neurological disorder that affects approximately one percent of the world population, are not well understood[1,2,3]. Using a mouse model of epilepsy, we show that seizures induce elevated expression of vascular cell adhesion molecules and enhanced leukocyte rolling and arrest in brain vessels mediated by the leukocyte mucin P-selectin glycoprotein ligand-1 (PSGL-1, encoded by *Selplg*) and leukocyte integrins $\alpha_4\beta_1$ and $\alpha_L\beta_2$. Inhibition of leukocyte-vascular interactions, either with blocking antibodies or by genetically interfering with PSGL-1 function in mice, markedly reduced seizures. Treatment with blocking antibodies after acute seizures prevented the development of epilepsy. Neutrophil depletion also inhibited acute seizure induction and chronic spontaneous recurrent seizures. Blood-brain barrier (BBB) leakage, which is known to enhance neuronal excitability, was induced by acute seizure activity but was prevented by blockade of leukocyte-vascular adhesion, suggesting a pathogenetic link between leukocyte-vascular interactions, BBB damage and seizure generation. Consistent with the potential leukocyte involvement in epilepsy in humans, leukocytes were more abundant in brains of individuals with epilepsy than in controls. Our results suggest leukocyte-endothelial interaction as a potential target for the prevention and treatment of epilepsy.

COMMENTARY

A flurry of recent papers has convincingly demonstrated that inflammation, blood–brain barrier (BBB) leakage, and seizures are etiologically linked. In human subjects and animal models, it was shown that cerebrovascular or inflammatory events can actually *cause* seizures (1,2). Accordingly, the study by Fabene et al. established that brain inflammation and BBB disruption are realistic triggers of seizures in a mouse model of status epilepticus.

There is substantial evidence supporting both CNS and intravascular inflammation as being seizure promoting or pro-epileptogenic. BBB damage is known to directly cause seizures (3,4) and to increase spontaneous seizure frequency (5). Blockade of CNS or systemic inflammation pathways (e.g., via inhibition of interleukin [IL]-1β signaling with IL1-receptor antagonist or via blockade of IL-1β production with caspase-1 inhibitors) reduces status epilepticus and seizure frequency (6,7–9). Glia, neurons, and endothelial cells express cytokines following seizures in experimental models (10), in human epileptogenic tissue (10–12), and after brain injury (13). These findings point to a prominent role for cytokines in the pathogenesis of seizures. Elucidation of the mechanisms

underlying the effects of cytokines in seizures highlights non-conventional modes of action involving direct effects on neuronal excitability (14–16) or a direct action on BBB integrity (3,9,17). Taken together, these findings establish a novel concept: altered brain function may result from parenchymal or extraparenchymal inflammatory signals, acting in concert or alone.

To shed light on the mechanisms by which BBB leakage may occur, Fabene and colleagues set out to test whether leukocyte–endothelial cell interactions are altered by seizures and whether they can, in turn, contribute to BBB leakage, seizure pathogenesis, and epilepsy. As occurred in the work by Marchi et al. (9,17), Fabene et al. focused mainly on peripheral inflammation using a mouse model of pilocarpine-induced status epilepticus, evolving to spontaneous seizures.

After the pilocarpine administration, endothelial cell activation was studied. Results showed vascular induction of leukocyte adhesion molecules, which are expressed at low levels under physiological conditions. Specific adhesion molecules reached their highest levels of expression within 1 week after pilocarpine challenge; the earliest time point at which induction was studied was 6 hours after the onset of seizures. The authors concluded that seizures induce the adhesion of circulating lymphocytes by upregulation of adhesion molecules.

A note of caution: the possibility of a direct effect of pilocarpine on leukocyte–vasculature interactions cannot be ruled out, as suggested by the significant upregulation of vascular cell adhesion molecule-1 (VCAM-1), even when status epilepticus was prevented pharmacologically. In this regard, recent evidence indicates that pilocarpine has a direct proinflammatory effect when endothelial and white blood cells (WBCs) are exposed to this drug in vitro, leading to production and release of IL-1β. This effect was confirmed using convulsant doses of pilocarpine, which when used in vivo induced elevated levels of serum IL-1β (17). Since these proinflammatory actions of pilocarpine in vivo occur shortly after drug injection and before the onset of status epilepticus, the possibility that the initial trigger of microvascular activation and consequent upregulation of adhesion molecules stems from pilocarpine itself should be considered.

In a second set of experiments, the authors report that leukocytes exposed to pilocarpine in vitro do not show increased expression of VCAM-1 receptors. However, this evidence does not exclude the fact that pilocarpine in vivo could enhance VCAM-1 expression in endothelial cells via IL-1β release by WBCs, with corresponding increased leukocyte adhesion (17). It is well known that IL-1β is a strong inducer of endothelial adhesion molecules (18,19). We can, therefore, envisage that the activation of WBCs by pilocarpine leads to a cascade of events causing the release of IL-1β and conse-

quent changes in BBB permeability; these effects synergistically potentiate direct CNS action of pilocarpine, leading to seizures (9,17). The requirement of BBB opening for pilocarpine proconvulsant effects was recently demonstrated by Uva et al. (20).

In a subsequent set of experiments that used the same pilocarpine model, Fabene et al. asked whether leukocyte adhesion contributes to status epilepticus. They showed prevention of status epilepticus in wild-type mice pretreated with an anti-α4–integrin antibody, which hampers the adhesion of leukocytes to VCAM-1 on endothelium. Since these adhesion molecules are only minimally expressed in naive mice and given the fact that the antibody cannot act in the absence of its target protein, these results indicate that VCAM-1 is likely upregulated before the onset of status epilepticus, possibly by pilocarpine itself. Thus, using this study protocol, the authors might have blocked or reduced status epilepticus (and, thus, neuropathology and epilepsy in mice) by preventing the mechanism by which pilocarpine can exert its convulsant action. To shed light on whether leukocyte adhesion is a specific mechanism associated with pilocarpine use, it will be crucial to evaluate adhesion molecule expression before the onset of pilocarpine-induced status epilepticus and to extend these results to other models of status epilepticus. In addition, it will be valuable to assess the seizure threshold after blockade of endothelial adhesion mechanisms. Such investigations would allow the sorting out of the contribution of adhesion mechanisms in the generation or prevention of seizures.

Fabene et al. asked whether leukocyte adhesion contributes to epileptogenesis. They induced status epilepticus for 1 hour, and then chronically treated mice with antibodies against adhesion molecules every other day for 20 days. The investigators found no effect of these treatments on acute status epilepticus, as reported for α4 integrin-specific antibody, but there was a significant reduction of daily spontaneous seizures 20 days post-status epilepticus, with no effect on timing of seizure onset or duration.

This interesting result supports previous findings showing that microvasculature alterations induced by status epilepticus and leading to BBB opening, significantly contribute to sustained hyperexcitability underlying spontaneous seizures (3,5,9,17,21). Previous evidence has also shown that when sustained seizure activity is induced in guinea-pig–isolated and CSF-perfused brain preparations, it stimulates expression of endothelial adhesion molecules (22). The induction of these molecules by seizures in the isolated brain may have been triggered by inflammatory reactions involving cytokines produced by perivascular astrocytes during epileptic activity (10). Once again, the effect of in vivo antiadhesion strategy, after status epilepticus, needs to be confirmed in a model of seizures in

which the primary convulsant stimulus has no direct peripheral proinflammatory actions to exclude the possible synergy between these peripheral effects and the effect of seizures in determining the pathological outcome.

Finally, Fabene et al. evaluated a population of human subjects to show that adhesion/extravasation of leukocytes is more commonly observed in epileptic patients than control subjects. The epileptic population studied consisted of postmortem autopsy samples derived from subjects with comorbidities ranging from hepatic failure to cardiac arrest, both known to lead to rapid disintegration of the BBB (2). It is, however, surprising that control subjects were devoid of WBC extravasation, given that the stated causes of death were ischemic in nature in eight of ten samples, as it is well known that brief ischemia or even normoxic–normoglycemic flow cessation cause inflammatory changes, leading to leukocyte adhesion and BBB disruption (2).

The relative epileptogenic contribution of inflammatory reactions intrinsic to the brain compared with those mediated by peripheral immune cells remains to be established. Unquestionably, BBB failure is the link between these two mechanisms. Human pathology studies show pronounced parenchymal cell expression of inflammatory markers, including IL-1β, cyclo-oxygenase-2 (COX-2), and complement factors, in all brain specimens evaluated so far for epilepsies of different etiologies (11,12,23,24). However, evidence for prominent recruitment of leukocytes in epileptogenic tissue has been reported in some epilepsies, such as tuberous sclerosis (12) and Rasmussen's encephalitis (25), but not in others, such as temporal lobe epilepsy (10).

In summary, this study reveals an interesting overlap among diseases associated with white matter, such as multiple sclerosis and epilepsy. Thus, it highlights the possibility that WBC interactions with brain vasculature, in the context of preexisting systemic inflammation, contribute to the pathogenesis of seizures, via disruption of BBB. The therapeutic potential of antiadhesion molecules to reduce the severity of epilepsy when administered after a period of status epilepticus is an attractive novel strategy. Similar antiepileptogenic effects from systemic treatments with α2-adrenoceptor antagonist, atipamezol (26), diazepam (27), and fluorofelbamate (28) significantly decreased the frequency of spontaneous recurrent seizures or their severity and afforded neuronal protection. Further elucidation of these mechanisms in additional clinically relevant experimental models is important, since this knowledge may open new therapeutic perspectives for seizures and epileptogenesis using anti-inflammatory drugs directed against parenchymal or peripheral inflammation that are already available for autoimmune chronic inflammatory diseases. This finding is supported by the recently reported effects of steroids in pediatric epilepsy (29).

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T-channels: Short-Term Up-Regulation Causes Long-Term Consequences in Epilepsy

Transcriptional Upregulation of Ca$_v$3.2 Mediates Epileptogenesis in the Pilocarpine Model of Epilepsy. Becker AJ, Pitsch J, Sochivko D, Opitz T, Staniek M, Chen CC, Campbell KP, Schoch S, Yaari Y, Beck H. *J Neurosci* 2008 Dec 3;28(49):13341–13353. In both humans and animals, an insult to the brain can lead, after a variable latent period, to the appearance of spontaneous epileptic seizures that persist for life. The underlying processes, collectively referred to as epileptogenesis, include multiple structural and functional neuronal alterations. We have identified the T-type Ca^{2+} channel Ca$_v$3.2 as a central player in epileptogenesis. We show that a transient and selective upregulation of Ca$_v$3.2 subunits on the mRNA and protein levels after status epilepticus causes an increase in cellular T-type Ca^{2+} currents and a transitional increase in intrinsic burst firing. These functional changes are absent in mice lacking Ca$_v$3.2 subunits. Intriguingly, the development of neuropathological hallmarks of chronic epilepsy, such as subfield-specific neuron loss in the hippocampal formation and mossy fiber sprouting, was virtually completely absent in Ca$_v$3.2$^{-/--}$ mice. In addition, the appearance of spontaneous seizures was dramatically reduced in these mice. Together, these data establish transcriptional induction of Ca$_v$3.2 as a critical step in epileptogenesis and neuronal vulnerability.

COMMENTARY

It is clear that ion channel dysfunction, or channelopathy, causes epilepsy in a number of relatively uncommon human genetic epilepsy syndromes. Channelopathy is suspected to be causative in the acquired epilepsies, which are considered to be far more common, but this assertion is far from proven. A number of studies in animal models of acquired epilepsy have found associated alterations in ion channels (1). However, is association causation? Minimal criteria to answer this question might include evidence that: 1) ion channel dysfunction occurs following a neural insult but preceding the onset of spontaneous seizures, 2) the alteration produces neuronal hyperexcitability, and 3) reversal of the alteration inhibits epileptogenesis or the development of the epileptic state. The recent paper by Becker