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Getting Excited Through Cyclin: A Role for Endothelial Cdk5 Signaling in Hippocampal Hyperexcitability

Endothelial Cdk5 Deficit Leads to the Development of Spontaneous Epilepsy Through CXCL1/CXCR2-Mediated Reactive Astrogliosis

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Blood–brain barrier dysfunction has been suggested to play an important role in epilepsy. However, the mechanism mediating the transition from cerebrovascular damage to epilepsy remains unknown. Here, we report that endothelial cyclin-dependent kinase 5 is a central regulator of neuronal excitability. Endothelial-specific Cdk5 knockout led to spontaneous seizures in mice. Knockout mice showed increased endothelial chemokine (C-X-C motif) ligand 1 (Cxcl1) expression, decreased astrocytic glutamate reuptake through the glutamate transporter 1 (GLT1), and increased glutamate synaptic function. Ceftriaxone restored astrocytic GLT1 function and inhibited seizures in endothelial Cdk5-deficient mice, and these effects were also reversed after silencing Cxcl1 in endothelial cells and its receptor chemokine (C-X-C motif) receptor 2 (Cxcr2) in astrocytes, respectively, in the CA1 by AAV transfection. These results reveal a previously unknown link between cerebrovascular factors and epileptogenesis and provide a rationale for targeting endothelial signaling as a potential treatment for epilepsy.[1]

Commentary

Cyclin-dependent kinases are a family of protein kinases that are activated by the binding of a cyclin molecule.[2] This group of kinases participates in the regulation of cell division and transcriptional mechanisms. Cyclin-dependent kinase 5 (CDK5) is mostly associated with the regulation of gene expression, cell differentiation and survival, and the development of new blood vessels (angiogenesis).[1-3] Because CDK5 signaling can modulate the stability of blood vessels,[1-3] and epilepsy is associated with vascular and blood–brain barrier (BBB) dysfunction,[4,5] the report by Liu et al recently published in the *Journal of Experimental Medicine* investigated the impact of endothelial CDK5 deletion on neuronal activity.[1] For this purpose, the authors created 3 different mouse models with endothelial-specific conditional CDK5 knockout (KO), Cdh5-Cre;$Cdk5^{f/f}$, Cdh5-CreERT2;$Cdk5^{f/f}$, and BR1-iCre-$Cdk5^{f/f}$. Following pharmacogenetic manipulations in these mice, the study assessed neural activity using electroencephalographic (EEG) recordings, seizure threshold and duration in response to pentylenetetrazol (PTZ), and neuronal action potential firing and spontaneous and miniature excitatory postsynaptic currents (EPSCs) in hippocampal and cortical neurons using whole-cell patch clamp recordings.

The study reports that all 3 different KO groups developed spontaneous recurrent seizures (SRS) that were evident by 8 weeks, and that the seizure frequency was significantly increased in 16- to 24-week-old Cdh5-Cre;$Cdk5^{f/f}$ mice. EEG and whole-cell recordings showed that the neuronal hyperexcitability occurred in the hippocampus, and not in the cortex, of the 3 different CDK5 KO mice. It is interesting that a similar hippocampal pathology was reported in all KO models despite differences in the extent of endothelial CDK5 messenger RNA (mRNA) loss between the groups ($\sim$100%, $\sim$75%, and $\sim$50% in Cdh5-Cre;$Cdk5^{f/f}$, BR1-iCre-$Cdk5^{f/f}$, and Cdh5-CreERT2;$Cdk5^{f/f}$ mice, respectively). However, the article does not provide an explicit rationale for the use of multiple CDK5 KO mouse models, or an interpretation on how endothelial CDK5 deficiency may directly result in the hippocampal-specific hyperexcitability, that would provide insights into potential underlying mechanisms. To begin understanding possible causes, information on the structure–function correlations between levels of endothelial CDK5 expression and the extent of neuronal activity in hippocampal and cortical neurons within the same animals would be useful.

Along with hippocampal hyperexcitability, endothelial CDK5 loss was associated with increased concentrations of extracellular glutamate in hippocampi of nonepileptic (4 week) and epileptic (16 week) Cdh5-Cre;$Cdk5^{f/f}$ mice compared to $Cdk5^{f/f}$ controls, which paralleled reactive astrogliosis as well as a decrease in astrocytic glutamate transporter 1

(GLT1)-mediated currents. These pathological features were attenuated by pharmacologically increasing GLT1 expression and function with the β-lactam antibiotic ceftriaxone (CEF) or with injections of AAV-GLT1-mCherry in 4-week-old nonepileptic Cdh5-Cre;Cdk5$^{f/f}$ mice, thereby suggesting a role for aberrant astrocytic GLT1 function in neuronal hyperexcitability in young Cdh5-Cre;Cdk5$^{f/f}$ mice. Note that CEF treatment following traumatic brain injury has previously been shown to reduce gliosis as well as post-traumatic seizures in rats by modulating GLT expression.[6] Therefore, as the seizure severity progresses with age in these mice, it would have been valuable to determine whether GLT1-enhancing treatments reduce SRS in the epileptic Cdh5-Cre;Cdk5$^{f/f}$ mice (8-24 week old). Information on the time points during the development and progression of epilepsy when the treatments are effective could lead to a deeper understanding of the mechanisms that can be targeted for disease modification.

Increased signaling through the chemokine (C-X-C motif) ligand 1 (CXCL1) and its receptor chemokine receptor 2 (CXCR2) as a candidate mechanism underlying the hippocampal hyperexcitability and astrocytic pathology in Cdh5-Cre;Cdk5$^{f/f}$ mice was identified using high-throughput mRNA sequencing. Silencing CXCL1 or CXCR2 signaling using shRNA, AAV-BR1-shCxcl1, or AAV2/9-GFAP-Cxcr2-RNAi reduced astrogliosis, increased GLT1 currents, reduced seizure onset and duration in response to PTZ, and decreased both the frequencies and amplitudes of sEPSC and mEPSC in hippocampal CA1 cells of 4-week-old Cdh5-Cre;Cdk5$^{f/f}$ mice. While these evidence suggest that endothelial CDK5 regulates CXCL1/CXCR2 signaling, their direct mechanistic association is not known. Based on the roles described for CDK5 in modulating blood vessel development,[1-3] we could speculate that endothelial-specific loss of CDK5 may promote BBB instability. A "leaky" BBB would allow extravasation of peripheral immune cells and associated molecules into the brain parenchyma and result in increased levels of CXCL1/CXCR2 signaling as well as inflammation. The study concluded that BBB damage was greater in 16-week-old Cdh5-Cre;Cdk5$^{f/f}$ mice after examining various markers of BBB leakage including Evans blue extravasation into the brain, exogenous tracer effusion, and perivascular deposits of fibrinogen along with various gap junction proteins. However, an objective quantification and statistical comparison was not reported between the 4- and 16-week-old mouse groups. Thus, it is still unclear to what extent the integrity of the BBB is different between 4- and 16-week old Cdh5-Cre;Cdk5$^{f/f}$ mice, and how it relates to the progression of seizure severity in these mice. Further analysis of BBB properties in the endothelial CDK5 KO mice would help define specific roles that endothelial-specific CDK5 signaling may play in the generation of epileptic networks.

A strength of this report by Liu et al is the comprehensive and straight forward step-by-step characterization of seizures, hippocampal hyperexcitability, and astroglial pathology along with potential underlying mechanisms using a myriad of electrophysiological and pharmacogenetic manipulations in endothelial-specific CDK5 KO mice. In addition, the finding that seizures and hippocampal hyperexcitability parallel astrocytic pathology and aberrant chemokine CXCL1/CXCR2 signaling is widely supported in the epilepsy literature. However, a limitation of this study is that an explicit rationale linking these findings (ie, GLT1, CXCL1 signaling, BBB dysfunction, and seizure pathology) to a central mechanistic hypothesis directly related to endothelial CDK5 signaling is not described or discussed. Thus, questions remain in regard to how an endothelial-specific CDK5 loss directly results in epileptogenesis and seizure development. Is this associated with a direct BBB dysfunction, or a direct CDK5-dependent signal transduction miscommunication between endothelial cells and other cell types such as astrocytes? A number of studies support that CDK5 signaling plays critical roles in the regulation of neuronal-specific functions including neuronal migration and synaptic plasticity, neuronal activity, and epileptogenic processes.[3,7] Additionally, evidence that reductions in CDK5 are associated with increased seizure susceptibility to chemoconvulsants such as PTZ and pilocarpine,[8] and that CDK5 participates in the mechanisms leading to kainate-induced synaptic damage in the hippocampus,[9] support that CDK5 signaling is important for regulating neuronal activity and epileptogenesis. Although more research is required to identify the direct mechanisms by which reductions in endothelial-specific CDK5 signaling result in seizures, these data suggest that CDK5 may be an interesting signaling molecule to further investigate as a potential novel therapeutic target in epilepsy.

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