

THE FRAGILE X MENTAL RETARDATION PROTEIN: A VALUABLE PARTNER IN THE BATTLE AGAINST EPILEPTOGENESIS

Correction of Fragile X Syndrome in Mice. Dölen G, Osterweil E, Rao BSS, Smith GB, Auerbach BD, Chattarji S, Bear MF. *Neuron* 2007;56:955–962. Fragile X syndrome (FXS) is the most common form of heritable mental retardation and the leading identified cause of autism. FXS is caused by transcriptional silencing of the *FMR1* gene that encodes the fragile X mental retardation protein (FMRP), but the pathogenesis of the disease is unknown. According to one proposal, many psychiatric and neurological symptoms of FXS result from unchecked activation of mGluR5, a metabotropic glutamate receptor. To test this idea we generated *Fmr1* mutant mice with a 50% reduction in mGluR5 expression and studied a range of phenotypes with relevance to the human disorder. Our results demonstrate that mGluR5 contributes significantly to the pathogenesis of the disease, a finding that has significant therapeutic implications for fragile X and related developmental disorders.

Limbic Epileptogenesis in a Mouse Model of Fragile X Syndrome. Qiu LF, Lu TJ, Hu XL, Yi YH, Liao WP, Xiong ZQ. *Cereb Cortex* 2009 in press. (doi:10.1093/cercor/bhn163) Fragile X syndrome (FXS), caused by silencing of the *Fmr1* gene, is the most common form of inherited mental retardation. Epilepsy is reported to occur in 20–25% of individuals with FXS. However, no overall increased excitability has been reported in *Fmr1* knockout (KO) mice, except for increased sensitivity to auditory stimulation. Here, we report that kindling increased the expressions of *Fmr1* mRNA and protein in the forebrain of wild-type (WT) mice. Kindling development was dramatically accelerated in *Fmr1* KO mice, and *Fmr1* KO mice also displayed prolonged electrographic seizures during kindling and more severe mossy fiber sprouting after kindling. The accelerated rate of kindling was partially repressed by inhibiting N-methyl-D-aspartic acid receptor (NMDAR) with MK-801 or mGluR5 receptor with 2-methyl-6-(phenylethynyl)-pyridine (MPEP). The rate of kindling development in WT was not effected by MPEP, however, suggesting that FMRP normally suppresses epileptogenic signaling downstream of metabotropic glutamate receptors. Our findings reveal that FMRP plays a critical role in suppressing limbic epileptogenesis and predict that the enhanced susceptibility of patients with FXS to epilepsy is a direct consequence of the loss of an important homeostatic factor that mitigates vulnerability to excessive neuronal excitation.

COMMENTARY

The fragile X mental retardation protein (FMRP) is an intracellular RNA-binding protein that serves to repress mRNA translation. It is widely expressed in many tissues but particularly abundant in the brain, especially in dendrites at glutamatergic synapses. Despite its name, this protein actually is absent in patients with fragile X syndrome, a condition that is the result of an inappropriate CGG trinucleotide repeat expansion of *FMR1*—the X-linked gene in humans that encodes the synthesis of FMRP. The phenotype of the *Fmr1* knockout mouse approximates well the human condition: memory deficits and learning difficulties, autism and epilepsy, altered body growth and macroorchidism are all features common to both. Similarly, neuropathological and electrophysiological studies have yielded analogous results in fragile X syndrome patients and knockout mice, revealing an abnormal abundance of immature long, thin dendritic spines in cortical neurons accompanied by aberrant synaptic plasticity (1,2).

How does the absence of FMRP result in this constellation of phenotypic abnormalities? FMRP is generally viewed as a protein synthesis inhibitor, targeting multiple dendritic mRNAs to

suppress their translation. Localized dendritic protein synthesis is a key requirement for the induction of long-term, activity-dependent synaptic plasticity, which is the fundamental physiologic mechanism for learning and memory and likely, an essential contributor to epileptogenesis (3). Nearly all the mRNAs that have been validated as targets for FMRP have been shown to be regulated by activation of group I metabotropic glutamate receptors (mGluRs) at some level (e.g., mRNA stability, dendritic transport, local translation, or protein expression) (4). Under normal conditions, group I mGluR activation will stimulate translation of FMRP target mRNAs and simultaneously will trigger ubiquitination and degradation of FMRP to de-repress these mRNA targets and allow, to some extent, induced protein synthesis to occur (5). However, should this mGluR-induced protein synthesis be allowed to take place unchecked, as would occur in the total absence of FMRP, severe sequelae may ensue, as demonstrated by the constellation of abnormalities seen in fragile X syndrome.

To test the hypothesis that fragile X syndrome is primarily the result of exaggerated group I mGluR-driven responses, Dölen and colleagues created a mouse that was crossbred to coexpress two genetic mutations, resulting in simultaneous absence of FMRP and 50% reduction in expression of mGluR5, a member of the group I mGluR family. They compared the behavior, anatomy, and physiology of this novel mutant mouse

to wild-type mice and to *Fmr1* knockout mice (i.e., lacking FMRP but with normal mGluRs). The authors performed an impressive array of experiments, testing synaptic plasticity in visual cortex, behavioral memory, audiogenic seizure susceptibility, dendritic spine density, basal protein synthesis in hippocampus, and prepubescent body growth—all of which are abnormal in the fragile X syndrome mouse model. Their findings demonstrated that each of these abnormalities was restored to control values in the fragile X hybrid with reduced mGluR5 expression, thereby, strongly validating the hypothesis. The only feature that was not rescued by the reduction of mGluR5 was macroorchidism: even when mGluR5 expression was completely eliminated, macroorchidism still appeared, suggesting a different mechanism for this phenotypic derangement (possibly driven by mGluR1, the other member of the group I mGluR family).

These data are remarkable, as they clarify the underlying mGluR-dependent pathogenesis of the disturbances expressed in fragile X syndrome. However, reversal of the phenotype in these experiments required reducing mGluR expression from the moment of conception. So, it still begs the question: how much benefit can blockade of mGluR5 provide after birth and development have taken place? Notably, mGluR5 antagonists do continue to provide therapeutic relief in fragile X syndrome patients, as shown by the suppressive effect of these agents on both seizures and behavioral abnormalities (6).

Rather than look at spontaneous or audiogenic seizures in fragile X syndrome as others had already done, Qiu and colleagues examined the development of kindling-induced seizures in the fragile X knockout mouse to determine whether there is a difference in epileptogenic propensity. Their control experiments, examining *Fmr1* mRNA and FMRP expression in kindled versus unkindled wild-type mice were not always substantiated, with many claims followed by the statement “data not shown.” Nevertheless, subsequent experiments in which the investigators compared kindling in wild-type and fragile X syndrome mice provide results with far-reaching implications.

When compared with wild-type animals, the knockout mice demonstrated a lower threshold for kindling. The mice expressed more robust seizures in response to fewer stimuli, which correlated with more severe mossy fiber sprouting on pathological examination. Although NMDA receptor antagonists could slow the progression of kindling in both control and knockout animals, only the fragile X mice were helped by MPEP, an mGluR5 antagonist. Hence, it appears that the enhanced sensitivity to kindling in the fragile X mice may be mediated by increased activation of mGluR5 or more robust downstream effects. These data suggest that exaggerated mGluR5 responsiveness may contribute to a predisposition for the development of epilepsy in this condi-

tion. The lowered threshold for the expression of robust seizures is consistent with studies performed in hippocampal slices in which application of GABA_A receptor antagonist, an agent that typically elicits only interictal discharges in control mice, evoked seizure-length discharges in *Fmr1* knockout mice, which could be suppressed with mGluR5 blockers (7,8).

Hence, in the case of mGluR-induced epileptogenesis, there appears to be a delicate balance between a potentially epileptogenic protein synthesized by mGluR5 activation and FMRP. In the normal neuronal network, Qiu et al. demonstrate that FMRP can limit the progression of epileptogenesis by preventing mGluR5 activation from playing a significant part in the process. However, it has also been shown that sufficient activation of mGluR5 can overcome the endogenous FMRP-mediated protection and elicit a long-lasting protein synthesis-driven enhancement of group I mGluR-mediated excitation in the hippocampal network, resulting in the persistent expression of seizure discharges (i.e., epileptogenesis) (9–11). Both Qiu and Dölen have shown that the converse is true as well: in the absence of FMRP, epileptogenesis is accelerated via enhanced mGluR5 participation; and, reduction of mGluR5 activation can compensate for the lack of FMRP in the fragile X mouse, restoring a near-normal phenotype.

The findings reported in these fragile X syndrome studies may have broad implications for the understanding of epileptogenic mechanisms in humans. Clinicians frequently encounter epileptogenesis in victims of head trauma or cortical strokes, for which there is typically a latency between the original insult and the onset of clinical seizures. One of the difficulties in the management of these patients is an inability to predict which patients will go on to develop epilepsy. It is logical to propose that the susceptibility to posttraumatic or poststroke epilepsy may be in part determined by the patient's endogenous level of FMRP expression or mGluR excitability. There are also different isoforms of FMRP whose functions as yet are undetermined. Perhaps, advances in genetic testing for variations in these molecules and their activity may one day allow targeted pharmacological therapies for those patients at greatest risk. These are exciting times for translational research in this arena; it may be that the development of truly antiepileptogenic therapies is finally within reach.

by Lisa R. Merlin, MD

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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 α_4 and L_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]-1 β signaling with IL1-receptor antagonist or via blockade of IL-1 β 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