MUTANT BATTEN DISEASE PROTEIN SAYS "NO" TO UNSATURATED FATS

CLN3P, the Batten's Disease Protein, Is a Novel Palmitoyl-Protein Δ-9 Desaturase Narayan SB, Rakheja D, Tan L, Pastor JV, Bennett MJ. *Ann Neurol* 2006;60(5):570–577. OBJECTIVE: Batten's disease, one of the most common recessively inherited, untreatable, neurodegenerative diseases of humans, is characterized by progressive neuronal loss and intraneuronal proteolipid storage. Although the gene for the disorder was cloned more than a decade ago, the function of the encoded protein, CLN3P, has not been defined thus far. METHODS: Sequence analysis using the Pfam server identified a low stringency match to a fatty acid desaturase domain in the N-terminal sequence of CLN3P. We developed a fatty acid desaturase assay based on measurement of desaturase products by gas chromatography/mass spectrometry. RESULTS: We show that CLN3P is a novel palmitoyl-protein Δ-9 desaturase, which converts membrane-associated palmitoylated proteins to their respective palmitoleated derivatives. We have further demonstrated that this palmitoyl-protein Δ-9 desaturase activity is deficient in *cln3*$^{-/-}$ mouse pancreas and is completely ablated in neuroblastoma cells by RNA inhibition. INTERPRETATION: We propose that palmitoyl-protein desaturation defines a new mechanism of proteolipid modification, and that deficiency of this process leads to the signs and symptoms of Batten's disease.

COMMENTARY

Although both clinicians and researchers now recognize epilepsy as a multifaceted central nervous system disease rather than simply an isolated seizure disorder (1), epileptologists give surprising little attention to progressive myoclonic epilepsy (PME) syndromes, neurodegenerative diseases that produce worsening neurological symptoms, as well as to myoclonic and tonic–clonic seizures (2). The neuronal ceroid lipofuscinoses (NCLs) comprise one subset of the PME syndromes, which in addition to myoclonic epilepsy, cause dementia, ataxia, early death, and except in the adult form, blindness. To date, NCL investigators have classified nine NCL subtypes, distinguished by age of onset, clinical features, pathology, and causative genes.

Juvenile NCL (JNCL), also called Batten disease, develops between ages 4 and 10 and typically starts with seizures along with rapid deterioration in vision, which is followed by motor, cognitive, and behavioral decline. Pathological features include neuronal death in the brain and retina; accumulation of the intracellular autofluorescent pigment, lipofuscin, and the presence of characteristic structures known as "fingerprint profiles," with or without "curvilinear profiles," identified via electron microscopy. The International Batten Disease Consortium cloned the Batten Disease gene, *CLN3*, in 1995 (3). The majority of patients possess a 1.02 kb deletion in both their *CLN3* genes, while the remainder possesses splice-donor site, frameshift, missense, or nonsense mutations (http://www.ucl.ac.uk/ncl/cln3.shtml) either in both genes or in one gene in conjunction with the 1.02 kb deletion in the other gene (i.e., compound heterozygotes).

Persaud-Sawin and Boustany demonstrated that cells lacking wildtype CLN3 protein (CLN3P) undergo apoptotic and autophagic cell death (4). However, despite intensive investigation, the mechanisms by which CLN3P prevents cell death remain unknown. One hypothesis, based on studies of the yeast CLN3P ortholog, holds that CLN3P helps acidify the lysosome and thus, cells lacking functional CLN3P have abnormal lysosomal function and lysosomal amino acid transport (5,6). Another hypotheses, inspired by identification of a characteristic motif in CLN3P's amino acid sequence, suggests that CLN3P functions to traffic sphingolipids from the Golgi to the plasma membrane and that deficient sphingolipid trafficking by mutant CLN3P impairs normal antiapoptotic signaling (7).

The results of the study by Narayan et al. suggest that CLN3P performs a previously unrecognized task and, moreover, that CLN3P represents a founding member of a new class of enzymes important for cell signaling. To discover possible CLN3P functions, Narayan et al. queried for proteins homologous to CLN3P within the Pfam database (http://pfam.janelia.org/), a database of protein families aligned by semimanual methods and thus is claimed by its curators to be especially sensitive to the identification of homologous proteins (8). The Pfam search revealed that CLN3P shared features with fatty acid desaturases, which are enzymes that insert double bonds at various positions within the long carbon chains of fatty acids. It must be emphasized that the Pfam search found only a very weak similarity between CLN3P and the fatty acid desaturase family;

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this similarity localized primarily to a 14 amino acid segment of CLN3P, starting at phenylalanine 41.

Given the very weak structural similarity between CLN3P and the fatty acid desaturase family, it appears remarkable that this study found desaturase activity associated with CLN3P. Narayan et al. characterized CLN3P-associated desaturase activity using lysates from neuroblastoma cells stably overexpressing CLN3P; desaturase enzyme kinetics were determined by nicotinamide adenine dinucleotide spectrophotometry and reaction products were identified by gas chromatography and mass spectroscopy. These methods revealed three particularly important results concerning CLN3P-containing lysates: (i) preferred substrates consisted of 16 carbon fatty acids (palmitate) not conjugated to coenzyme A, but rather conjugated to either cysteine or the Ras protein; (ii) the desaturase inserted a single double bond between the ninth and tenth carbon atoms, as numbered from the carbonyl carbon (i.e., a Δ-9 desaturase); and (iii) small inhibitory RNA "knock-down" of CLN3P mRNA substantially inhibited desaturase activity. In addition, Narayan et al. demonstrated that both pancreas and brain lysates obtained from *Cln3* knockout transgenic mice lacked substantial desaturase activity. These results certainly suggested that CLN3P participated in Δ-9 desaturation of palmitoylated proteins. However, the experiments conducted by the investigators did not directly demonstrate that the CLN3P protein, itself, contained the catalytic active site. Despite the sequence homology of CLN3P and the fatty acid desaturase family, these experiments did not exclude the possibility that another, unidentified protein in the lysate performed the catalytic activity and that CLN3P simply acted as a necessary associated protein. In fact, although lymphoblasts express CLN3P (6), Narayan et al. were unable to measure Δ-9 desaturase activity in lymphoblast lysates, a result that could suggest that a protein other than CL3P engaged in catalysis. Hopefully, future studies will characterize the Δ-9 desaturase activity of purified CLN3P and define the catalytic active site.

How could the lack of CLN3P Δ-9 desaturase activity result in neuronal death? While previously characterized Δ-9 desaturases act upon coenzyme A conjugated fatty acids (9), this investigation showed that CLN3P-associated desaturase activity preferentially used palmitoylated proteins as substrates and thus, in fact, defined a new enzyme class. As Narayan et al. discuss, palmitoylation functions as an important reversible posttranslational modification to target signaling molecules to specialized membrane domains, the so-called "lipid rafts" (10). Thus, it would be expected that alteration of the conjugated palmitate by the creation of the Δ-9 double bond could modulate the targeting of these signaling molecules. Therefore, further insight into the role of CLN3P-associated desaturation activity may provide important clues not only to Batten disease pathogenesis but to cellular neurophysiology as well.

by Martin J. Gallagher, MD, PhD

References

1. Gilliam FG, Mendiratta A, Pack AM, Bazil CW. Epilepsy and common comorbidities: improving the outpatient epilepsy encounter. *Epileptic Disord* 2005;7(Suppl 1):27–33.
2. Shahwan A, Farrell M, Delanty N. Progressive myoclonic epilepsies: a review of genetic and therapeutic aspects. *Lancet Neurol* 2005;4:239–248.
3. The International Batten Disease Consortium. Isolation of a novel gene underlying Batten disease, CLN3. The International Batten Disease Consortium. *Cell* 1995;82:949–957.
4. Persaud-Sawin DA, Boustany RM. Cell death pathways in juvenile Batten disease. *Apoptosis* 2005;10:973–985.
5. Pearce DA, Ferea T, Nosel SA, Das B, Sherman F. Action of BTN1, the yeast orthologue of the gene mutated in Batten disease. *Nat Genet* 1999;22:55–58.
6. Ramirez-Montealegre D, Pearce DA. Defective lysosomal arginine transport in juvenile Batten disease. *Hum Mol Genet* 2005;14:3759–3773.
7. Persaud-Sawin DA, McNamara JO, Rylova S, VanDongen A, Boustany RM. A galactosylceramide binding domain is involved in trafficking of CLN3 from Golgi to rafts via recycling endosomes. *Pediatr Res* 2004;56:449–463.
8. Sonnhammer EL, Eddy SR, Durbin R. Pfam: a comprehensive database of protein domain families based on seed alignments. *Proteins* 1997;28:405–420.
9. Nakamura MT, Nara TY. Structure, function, and dietary regulation of D6, D5, and D9 desaturases. *Annu Rev Nutr* 2004;24:345–376.
10. Resh MD. Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. *Biochim Biophys Acta* 1999;1451:1–16.

No Calm before the Storm: Reduced GABA Inhibition Precedes Seizures in Tish Rats

GABAergic Synaptic Inhibition Is Reduced before Seizure Onset in a Genetic Model of Cortical Malformation Trotter SA, Kapur J, Anzivino MJ, Lee KS. *J Neurosci* 2006;26(42):10756–1067. Malformations of the neocortex are a common cause of human epilepsy; however, the critical issue of how disturbances in cortical organization render neurons epileptogenic remains controversial. The present study addressed this issue by studying inhibitory structure and function before seizure onset in the telencephalic internal structural heterotopia (tish) rat, which is a genetic model of heightened seizure susceptibility associated with a prominent neocortical malformation. Both normally positioned (normotopic) and misplaced (heterotopic) pyramidal neurons in the tish neocortex exhibited lower resting membrane potentials and a tendency toward higher input resistance compared with pyramidal neurons from control brains. GABAergic synaptic transmission was attenuated in the tish cortex, characterized by significant reductions in the frequency of spontaneous IPSCs (sIPSCs) and miniature IPSCs recorded from pyramidal neurons. In addition, the amplitudes of sIPSCs were reduced in the tish neocortex, an effect that was more profound in the normotopic cells. Immunohistochemical assessment of presynaptic GABAergic terminals showed a reduction in terminals surrounding pyramidal cell somata in normotopic and heterotopic tish neocortex. The attenuation of inhibitory innervation was more prominent for normotopic neurons and was associated with a reduction in a subset of GABAergic interneurons expressing the calcium-binding protein parvalbumin. Together, these findings indicate that key facets of inhibitory GABAergic neurotransmission are disturbed before seizure onset in a brain predisposed to developing seizures. Such alterations represent a rational substrate for reduced seizure thresholds associated with certain cortical malformations.

COMMENTARY

Disorders of cortical development comprise one of the most frequent causes of epilepsy. Such malformations include congenital errors in neuron proliferation, migration, and synaptogenesis. Numerous clinical syndromes of cortical malformation have been identified, and the genetic basis for some of these syndromes has been determined (1). Nevertheless, the mechanisms by which cortical malformations lead to epileptogenesis remain incompletely understood. Animal models have been used to study the mechanisms by which epilepsy can arise in dysplastic brain (2,3). Several animal models employ exogenous insults to the brain—such as freeze lesions, cranial irradiation, and prenatal exposure to toxins (e.g., methylazoxymethanol)—to produce disrupted cellular development and heightened excitability. While these models clearly provide mechanistic information about malformation-induced epileptogenesis, a naturally occurring or genetic malformation is more relevant clinically.

In contrast to lesion-induced models, the telencephalic internal structural heterotopia (tish) rat entails a genetic mutation that leads to both a specific cortical malformation (heterotopic band of unlaminated gray matter subjacent to a thinned but appropriately laminated normotopic cortex) and to the occurrence of spontaneous seizures at a certain age of development (about postnatal day 30 [P30]) (4). The seizures in tish rats appear to originate in the normotopic cortex overlying the heterotopic band, rather than in the heterotopia itself (5). However, both normotopic and heterotopic cortex receive inputs from appropriate cortical and subcortical targets and send projections to other cortical regions (6); therefore, the heterotopic neurons also might create hyperexcitable circuits within neocortex. The tish model overrides some disadvantages of the lesion-induced variety and exhibits some features similar to the human syndrome of subcortical band heterotopia. However, human subcortical band heterotopia is usually caused by an X-linked mutation of the doublecortin gene *DCX*, whereas the gene mutation in the tish rat is autosomal recessive and has not yet been identified. Therefore, these two syndromes both produce heterotopic bands of abnormal neurons and spontaneous seizures but have different genetic bases.

Understanding of the pathophysiological basis of hyperexcitability and, hence, of seizure propensity has been based on the simple concept that seizures arise from increased excitation, decreased inhibition, or both. While this conceptualization now is considered to be oversimplified (e.g., overexpression of GABAergic inhibition sometimes causes enhanced excitation and seizures (7)), it remains a valuable construct for approaching epilepsy pathogenesis. In this regard, the paper by Trotter et al. examines possible mechanisms for cortical hyperexcitability in the tish mutant rat before the onset of spontaneous seizures by carefully dissecting potential alterations of GABAergic neurotransmission. Lee, Chen, Schottler, and colleagues have characterized the histological features and physiological aspects of the tish mutant rat in detail in previous reports (4–6). They now investigate some specific pathophysiological features that might lead to epileptogenesis in this model.

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Electrophysiology (whole cell recordings) and biocytin staining of large layer V pyramidal cells and immunochemistry for interneurons were compared among normal cortex of Sprague-Dawley rats and normotopic and heterotopic cortex of tish rats of Sprague-Dawley background, all at P15. Membrane properties and firing patterns did not differ significantly among the groups. However, the frequency and amplitude of action-potential–dependent spontaneous inhibitory postsynaptic currents (sIPSCs) were reduced in tish normotopic cortex compared to control cortex, whereas action-potential–independent miniature IPSCs (mIPSCs) were similar in each group. These results suggest that, in tish brain, reduced sIPSCs are due to diminished multiquantal, multiterminal release events rather than to smaller quantal size. Increasing the release probability by exposure to low Mg^{2+}/high Ca^{2+} did not fully restore inhibition in the tish normotopic cortex, as assessed by sIPSC frequency and amplitude. Furthermore, calcium channels (N subtype) responsible for vesicle release from cortical interneurons are present and functional in tish brain, and there is no shift in calcium channel subtype (from N to P/Q), based on experiments with specific calcium channel blockers. Suspecting a presynaptic localization for the inhibition defect in tish brain, the investigators then examined the intensity and distribution of glutamate decarboxylase (GAD-65) immunoreactivity and found fewer GABAergic terminals innervating tish normotopic layer V neurons than cells from control cortex. In tish brains, there were fewer parvalbumin-containing interneurons, suggesting a decrease in this specific inhibitory interneuron subtype, as also seen in other malformation models (8). In tish heterotopic cortex, each of the above findings also occurred but less dramatically, consistent with the observation that normotopic cortex is the primary driver of seizures in tish rats.

The authors conclude that hyperexcitability in tish rat brain, at least in part, is due to a deficit in a specific subtype of interneuron that ordinarily provides dense innervation of neocortical pyramidal output neurons. The reduced inhibitory drive onto these principal neurons represents a plausible structural and neurochemical explanation for heightened seizure predisposition in this model of cortical dysplasia. Other mechanisms, such as $GABA_A$ receptor subunit stoichiometry, alterations in GABA reuptake, and pathology of other interneuron subtypes, might also be involved. The key step now is to show directly that this reduced inhibition leads to seizures and to determine whether it is possible to modify epileptogenesis by enhancing GABA function in these mutants. It is also critical to learn how the calming effects of GABAergic inhibition change over time as the seizure ("electrical storm") approaches and hits. This model provides an excellent substrate in which to pursue such questions and offers insights into epilepsy in human syndromes of cortical dysplasia.

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References

1. Guerrini R, Filippi T. Neuronal migration disorders, genetics, and epileptogenesis. *J Child Neurol* 2005;20:287–299.
2. Baraban SC. Epileptogenesis in the dysplastic brain: a revival of familiar themes. *Epilepsy Curr* 2001;1:6–11.
3. Schwartzkroin PA, Roper SN, Wenzel HJ. Cortical dysplasia and epilepsy: animal models. In: Binder DK, Scharfman HE, eds. *Recent Advances in Epilepsy Research*. New York: Kluwer Academic/Plenum Publishers, 2004:145–174.
4. Lee KS, Schottler F, Collins JL, Lanzino G, Couture D, Rao A, Hiramatsu K, Goto Y, Hong SC, Caner H, Yamamoto H, Chen ZF, Bertram E, Berr S, Omary R, Scrable H, Jackson T, Goble J, Eisenman L. A genetic animal model of human neocortical heterotopia associated with seizures. *J Neurosci* 1997;17:6236–6242.
5. Chen ZF, Schottler F, Bertram E, Gall CM, Anzivino MJ, Lee KS. Distribution and initiation of seizure activity in a rat brain with subcortical band heterotopia. *Epilepsia* 2000;41:493–501.
6. Schottler F, Couture D, Rao A, Kahn H, Lee KS. Subcortical connections of normotopic and heterotopic neurons in sensory and motor cortices of the tish mutant rat. *J Comp Neurol* 1998;395:29–42.
7. Cossart R, Bernard C, Ben-Ari Y. Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. *Trends Neurosci* 2005;28:108–115.
8. Roper SN, Eisenschenk S, King MA. Reduced density of parvalbumin- and calbindin D28-immunoreactive neurons in experimental cortical dysplasia. *Epilepsy Res* 1999;37:63–71.

How Does the Balance of Excitation and Inhibition Shift during Epileptogenesis?

Cell Domain-Dependent Changes in the Glutamatergic and GABAergic Drives during Epileptogenesis in the Rat CA1 Region El-Hassar L, Milh M, Wendling F, Ferrand N, Esclapez M, Bernard C. *J Physiol* 2007;578(Pt 1):193–211.

An increased ratio of the glutamatergic drive to the overall glutamatergic/GABAergic drive characterizes the chronic stage of temporal lobe epilepsy (TLE), but it is unclear whether this modification is present during the latent period that often precedes the epileptic stage. Using the pilocarpine model of TLE in rats, we report that this ratio is decreased in hippocampal CA1 pyramidal cells during the early phase of the latent period (3–5 days postpilocarpine). It is, however, increased during the late phase of the latent period (7–10 days postpilocarpine), via cell domain-dependent alterations in synaptic current properties, concomitant with the occurrence of interictal-like activity in vivo. During the late latent period, the glutamatergic drive was increased in somata via an enhancement in EPSC decay time constant and in dendrites via an increase in EPSC frequency and amplitude. The GABAergic drive remained unchanged in the soma but was decreased in dendrites, since the drop off in IPSC frequency was more marked than the increase in IPSC kinetics. Theoretical considerations suggest that these modifications are sufficient to produce interictal-like activity. In epileptic animals, the ratio of the glutamatergic drive to the overall synaptic drive was not further modified, despite additional changes in synaptic current frequency and kinetics. These results show that the global changes to more glutamatergic and less GABAergic activities in the CA1 region precede the chronic stage of epilepsy, possibly facilitating the occurrence and/or the propagation of interictal activity.

COMMENTARY

The paper by El-Hassar and coworkers analyzed changes in excitatory (glutamatergic) and inhibitory (GABAergic) synaptic input that follows pilocarpine-induced status epilepticus; the study focused on reduced inhibitory postsynaptic currents (IPSCs) and increased excitatory postsynaptic currents (EPSCs) at the somata and apical dendrites during the early and late phases of the seizure-free latent period. The analysis of reduced inhibition is based on several lines of evidence from previous studies concerning the loss of specific types of GABAergic interneurons after kainate- or pilocarpine-induced status epilepticus (1,2). Similarly, the analysis of increased excitation relates to synaptic reorganization, likely arising from the onset of axonal sprouting and an increase in recurrent excitatory circuitry (3,4), which probably begins to occur within a few days after injury. The experiments used measurements of the amplitude, frequency, rise time, decay time, and charge transfer for both spontaneous IPSCs and EPSCs. By using whole-cell recording from visually identified pyramidal cells, the authors also were able to analyze the synaptic inputs to the dendrites versus the somata. Thus, these data describe the time-dependent changes in GABAergic and glutamatergic inputs to dendrites and somata during the early stages of epileptogenesis. The changes in the characteristics of the postsynaptic currents, as measured in vitro, reflect spontaneous release of transmitter from axons cut during brain slicing (i.e., presumed to have no action potentials); activity-dependent transmitter release from neurons present in the slice; and the effects of different synaptic inputs on those axon terminals and neurons in the recorded slice.

This paper aims to address the important conceptual issue of epileptogenesis, which is often defined as the changes in intrinsic and synaptic mechanisms that occur during the latent period between brain injury and the onset of spontaneous recurrent seizures. The authors have separated the latent period in the pilocarpine model into early and late epochs and have contrasted the data from these time epochs with the chronic phase of spontaneous recurrent seizures. As expected, the recordings reveal that changes are present within a few days after status epilepticus; further changes were detected within a few additional days, during the latent period before seizures generally begin. The data support the previous hypotheses that: (a) dendritic inhibition is reduced from the loss of dendritically projecting neurons, (b) somatic inhibition is increased from the loss of inhibitory input to somatically projecting interneurons, and (c) excitation is increased from formation of new recurrent excitatory circuits (5). As with many studies on synaptic reorganization, however, the data and the potential circuit mechanisms are more complicated than is immediately obvious.

The experiments by El-Hassar et al. focused on spontaneous IPSCs and EPSCs recorded in normal solutions, so that both inhibitory and excitatory mechanisms were pharmacologically intact. This protocol is in contrast to some other published reports in which GABAergic inhibition was studied by pharmacologically blocking glutamatergic excitation (6) or glutamatergic inputs were analyzed by blocking GABAergic transmission with $GABA_A$ and $GABA_B$ receptor antagonists (7). The former approach has the advantage that the tissue is more similar to the intact animal; however, the potential disadvantage is that the effects of epilepsy-associated alterations of one transmitter

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system will impact the analysis of another system, and vice versa. For example, recordings of spontaneous EPSCs in CA1 pyramidal cells in normal solution reflect spontaneous transmitter release from all of the cut and uncut glutamatergic axons (i.e., miniature EPSCs) as well as from those glutamatergic neurons that are intact and spontaneously active. The activity of the latter neurons, in turn, will depend on their glutamatergic and GABAergic inputs, which may or may not be intact. This experimental system, therefore, is more complete but also more complicated than one in which the glutamatergic and GABAergic transmitter systems have been isolated. Thus, these experiments greatly reduce the complexity that is inherent in an intact animal preparation and also bypass the interpretational problems associated with experiments involving electrical stimulation, which are more complex than commonly appreciated.

A concept elaborated in this report is that GABA-mediated inhibition is decreased immediately after pilocarpine-induced status epilepticus, presumably as a result of the loss of specific interneurons (1–3) but also potentially because of other alterations arising from direct status-epilepticus–induced changes in $GABA_A$-receptor–mediated mechanisms. These changes, and potentially others, are hypothesized to lead to an increased propensity for generation of interictal spikes, which may be involved in the process of epileptogenesis (8). It is noteworthy that the isolated CA1 area does not typically generate spontaneous all-or-none epileptiform bursts reminiscent of interictal spikes in slices from normal animals treated with pharmacological agents that block $GABA_A$ receptors. The authors did not report bursts in any of the different types of slices (i.e., from sham controls; pilocarpine-treated during the latent period; or pilocarpine-induced chronically epileptic), although interictal spikes were recorded in freely behaving animals during the latent period before chronic spontaneous recurrent seizures (9). Although the authors included modeling studies that suggested that the detected alterations could account for the generation of interictal spikes, it also is possible that the generation of these events in the CA1 area could arise from abnormalities in other regions, such as the CA3 area, and be projected to the CA1 area. It is likely, therefore, that the changes described here—particularly the loss of GABAergic interneurons—occur in many areas, including the CA3 area, dentate gyrus, and entorhinal cortex and collectively could lead to the propensity to generate interical spikes, possibly over relatively large areas.

El-Hassar et al. found a particularly large increase in spontaneous glutamatergic input to the dendrites that was not evident in somatic recordings. While the origins of the differences between dendritic versus somatic recordings have been questioned, recent studies, using voltage-sensitive dye imaging technology, demonstrated a similar increase in glutamatergic input to the dendrites of CA1 pyramidal cells (10). These findings raise the possibility that key shifts in the balance between excitation and inhibition may take place in the dendrites. Dendritic alterations are particularly interesting because, as El-Hassar et al. speculate, the shift in balance of excitation and inhibition may well underlie the generation of interictal spikes and because the paroxysmal depolarizing shift in membrane potential (considered to underlie the interictal spike) has long been thought to depend on giant excitatory postsynaptic potentials and calcium spikes, both of which appear to originate in the dendrites.

The paper of El-Hassar and coworkers highlights several important points. First, analyses of synaptic inputs to hippocampal pyramidal cells (or any type of neuron) in tissue from an animal model of epilepsy can be quite complicated; although in vitro studies can simplify the analyses of synaptic mechanisms, the details of the experimental protocols may have important effects on the results. Second, a reduction of GABAergic input from a partial loss of specific interneurons is a common feature of the short-term effects of status epilepticus, and this effect has been detected in many studies as a reduction in the frequency of spontaneous IPSCs (and miniature IPSCs). Third, a reduction in $GABA_A$-mediated inhibition is a common method of inducing large and prolonged burst discharges (i.e., a few hundred milliseconds), which is a model for the interictal spike. Fourth, events that resemble prolonged synchronous bursts and interictal spikes often precede seizure-like events in vitro and frank seizures in vivo. The connections among these phenomena will require further investigations in order to link both interictal spikes and seizures associated with epilepsy to molecular, cellular, and network mechanisms.

by F. Edward Dudek, PhD, and Kevin J. Staley, MD

References

1. Morin F, Beaulieu C, Lacaille JC. Selective loss of GABA neurons in area CA1 of the rat hippocampus after intraventricular kainate. *Epilepsy Res* 1998;32:363–369.
2. Obenaus A, Esclapez M, Houser CR. Loss of glutamate decarboxylase mRNA-containing neurons in the rat dentate gyrus following pilocarpine-induced seizures. *J Neurosci* 1993;13:4470–4485.
3. Esclapez M, Hirsch JC, Ben Ari Y, Bernard C. Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. *J Comp Neurol* 1999;408:449–460.
4. Shao LR, Dudek FE. Increased excitatory synaptic activity and local connectivity of hippocampal CA1 pyramidal cells in rats with kainate-induced epilepsy. *J Neurophysiol* 2004;92:1366–1373.
5. Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, De Felipe J, Ben-Air Y, Esclapez M, Bernard C. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nature Neurosci* 2001;4:52–62.
6. Shao L-R, Dudek FE. Changes in mIPSCs and sIPSCs after kainate treatment: evidence for loss of inhibitory input to dentate granule cells and possible compensatory responses. *J Neurophysiol* 2005;94:952–960.

7. Wuarin JP, Dudek FE. Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. *J Neurosci* 1996;16:4438–4448.
8. Staley KJ, Dudek FE. Interictal spikes and epileptogenesis. *Epilepsy Curr* 2006;6:199–202.
9. Hellier JL, Patrylo PR, Dou P, Nett M, Rose GM, Dudek FE. Assessment of inhibition and epileptiform activity in the septal dentate gyrus of freely behaving rats during the first week after kainate treatment. *J Neurosci* 1999;19:10053–10064.
10. Ang CW, Carlson GC, Coulter DA. Massive and specific dysregulation of direct cortical input to the hippocampus in temporal lobe epilepsy. *J Neurosci* 2006;26:11850–11856.

On Demand Up-regulation of Therapeutic Genes in the Brain: Fiction or Reality?

Enhancing $GABA_A$ Receptor α 1 Subunit Levels in Hippocampal Dentate Gyrus Inhibits Epilepsy Development in an Animal Model of Temporal Lobe Epilepsy Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR. *J Neurosci* 2006;26(44):11342–11346. Differential expression of $GABA_A$ receptor (GABR) subunits has been demonstrated in hippocampus from patients and animals with temporal lobe epilepsy (TLE), but whether these changes are important for epileptogenesis remains unknown. Previous studies in the adult rat pilocarpine model of TLE found reduced expression of GABR α1 subunits and increased expression of α4 subunits in dentate gyrus (DG) of epileptic rats compared with controls. To investigate whether this altered subunit expression is a critical determinant of spontaneous seizure development, we used adeno-associated virus type 2 containing the α4 subunit gene (GABRA4) promoter to drive transgene expression in DG after status epilepticus (SE). This novel use of a condition-dependent promoter upregulated after SE successfully increased expression of GABR α1 subunit mRNA and protein in DG at 1–2 weeks after SE. Enhanced α1 expression in DG resulted in a threefold increase in mean seizure-free time after SE and a 60% decrease in the number of rats developing epilepsy (recurrent spontaneous seizures) in the first 4 weeks after SE. These findings provide the first direct evidence that altering GABR subunit expression can affect the development of epilepsy and suggest that α1 subunit levels are important determinants of inhibitory function in hippocampus.

COMMENTARY

The development of gene transfer techniques in vivo has allowed for modification of cell phenotype either by ectopic expression of foreign peptides/proteins or by increased expression or suppression of endogenous molecules. These approaches can be exploited to study the function of specific genes in a variety of tissues and organs or to treat or eliminate the causes of some diseases. The delivery of genes into the CNS provides a special challenge because of the blood–brain barrier (which precludes entry of various xenobiotics, thus impairing noninvasive routes of gene delivery); the inaccessibility of various deep brain regions; and the nonmitotic nature of most cells, which prevents use of gene transduction methods that require DNA integration. Neurotropic adeno-associated viral (AAV) vectors represent one of the best tools for gene delivery to the CNS, because they provide long-term neuronal expression in a controllable manner and are nonpathogenic. Thus far, clinical applications for gene transfer to the CNS have been developed for Parkinson's and Alzheimer's disease (http://www.nlm.nih.gov/medlineplus/genesandgenetherapy.html), while a protocol for neuropeptide Y gene transfer in epilepsy is under evaluation at the U.S. Food and Drug Administration.

Two distinct goals are relevant to the design of a gene therapy approach for epilepsy: first, to achieve an anticonvulsant effect (to suppress spontaneous recurrent seizures), and second to provide an antiepileptogenic effect (to prevent the development of epilepsy). Most antiepileptic drugs control neuronal hyperexcitability by decreasing excitatory neurotransmission or by enhancing inhibitory neurotransmission (1). A decrease in GABA-mediated inhibition resulting from molecular changes in the $GABA_A$-receptor complex is one of the maladaptive alterations in brain injury that can contribute to epileptogenesis. Accordingly, reduced expression of $GABA_A$-receptor α1 subunits concomitant with increased α4-subunit was found in the dentate gyrus of surgical specimens of temporal lobe epilepsy patients (2,3). These receptor subunit modifications also occur in adult rats experiencing status epilepticus (SE) (2), while opposite changes were observed in neonatal rats undergoing SE but not developing epilepsy (4).

Using this information, Raol and collaborators tested the hypothesis of a causal link between changes in $GABA_A$-receptor α1 subunit and the development of epilepsy. They introduced the $GABA_A$ receptor α1 subunit gene (GABR α1) into the

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rat hippocampus, where it remained quiescent until an injury-dependent stimulus (i.e., SE) was provided. The human minimal $GABA_A$-receptor $\alpha 4$-subunit gene promoter was applied to drive the transcription of the *GABRA1* gene (encoding GABR $\alpha 1$) in an AAV vector cassette, since it previously was identified that this promoter activity is upregulated in dentate gyrus after SE (5). The promoter chosen in the work of Raol et al., however, did not provide long-term expression of the transgene, as upregulation of the GABR $\alpha 1$ subunit was observed in the hippocampus at 14 days after SE, rapidly declining thereafter. The reason for this transient effect still is unresolved but several potential mechanisms exist, including promoter silencing or transduced cell loss. Transcriptional down-regulation of the transgene from depletion of transcriptional factors (e.g., brain-derived neurotrophic factor or early growth response factor 3) appears unlikely since depletion should have, but did not, affected the levels of the GABR $\alpha 4$ subunit. Influences of the immune system also are unlikely because the inhibitory effect of anti-AAV antibodies on transduction in the brain is very limited (6).

Other promoters, such as the cytomegalovirus/chicken β-actin promoter, can provide AAV-mediated transgene expression for up to 9 months in rodents, particularly when regulatory elements are included to increase the steady-state levels of the mRNA (7). However, this promoter cannot be induced by SE, but permits stable enhanced expression of the transgene independent of the injurious event. Another approach is regulation of the promoter activity by including a tetracycline-sensitive cassette, which has been successfully used to modulate the AAV-mediated galanin (*GAL*) gene expression in the rat brain (8). In this study, the elevated expression of galanin, an anticonvulsant peptide, in the inferior colliculus increases the threshold of wild-running seizures triggered by local electrical stimulation. However, when doxycycline, which binds to the tetracycline cassette, was added to drinking water, *GAL* gene transcription and consequent protein synthesis were blocked and the threshold to seizures decreased to baseline levels within 1 week. This effect was reversed upon doxycycline removal, highlighting the possibility of switching on or off a specific gene.

The choice of the vector serotype for transgene delivery is important since the viral capsid protein composition can influence vector spread in the injected tissue and the type of cells transduced (9,10); the type and extent of the cells transfected will determine the functional outcome. Eleven distinct AAV serotypes have been isolated to date but the transduction properties of the vast majority have not yet been characterized in the brain. AAV vectors generally have preferential uptake by neurons (15), indicating that the lack, or minimal expression in glia, is not due to absence of promoter activity. Raol et al. chose the AAV5 serotype capsid because by binding to the platelet-derived growth factor-α receptor it preferentially targets neurons.

Raol and colleagues demonstrate that the *GABRA1* gene was transcribed following AAV vector injection by measuring the corresponding mRNA levels; additionally, by western blot analysis they showed that the corresponding protein was synthesized in tissue. Also important is to determine whether the protein is expressed in the proper cell compartment (e.g., at the cell membrane for a receptor protein) and to identify, by immunohistochemistry, the cell populations in the hippocampus where GABR $\alpha 1$ subunit is increased, as the location will determine the functional outcome. An elegant study by Haberman et al. showed that the NMDA-receptor gene antisense sequence leads to down-regulation of the receptor protein in the rat collicular cortex—either in GABA interneurons or in excitatory output neurons (depending on the AAV vector cassette promoter used), resulting in a decrease or increase in focal seizure sensitivity, respectively (11). Therefore, although neurotransmitter receptors and ion channels represent an obvious target for inhibition of seizures, a detailed knowledge of the expression patterns in the injected area is required so that the therapeutic strategy can be properly designed to avoid an undesired increase in seizure sensitivity.

Raol et al. induced SE in rats 2 weeks after the vector injection to evaluate whether epileptogenesis was impaired and found no difference in the amount of pilocarpine needed to provoke seizures or SE onset between controls and rats injected with the vector. This is a crucial finding since SE represents the injurious event leading to epilepsy and, theoretically, onset should not be altered by the experimental conditions. An analysis of the EEG characteristics during epileptic activity and quantification of relevant parameters could demonstrate unequivocally that SE was unaltered by experimental manipulations. The authors found a significantly lower percentage of AAV-*GABRA1* injected rats developing spontaneous behavioral seizures within 4 weeks of SE induction as compared to control rats. EEG analysis was performed on a subgroup of rats, revealing that behavioral seizures were invariably associated with EEG seizures. The authors acknowledge that the conservative interpretation of their results is that overexpression of the GABR $\alpha 1$ subunit of the receptor in the hippocampus retards the occurrence of spontaneous seizures in a substantial population of rats. However, whether transgene overexpression produces an anticonvulsant effect or a true antiepileptogenic effect remains unclear and requires a longer follow-up study of spontaneous seizures, possibly including a viral vector construct that produces a more persistent elevation of the transgene. In addition to the effects on seizures, these authors analyzed the presence of side effects in rats overexpressing the transgene and found that 30% of the rats showed sedation, anorexia, and weight loss, raising the concern that brain functions may be significantly affected when gene expression is modified.

Numerous studies now have demonstrated that gene therapy interventions in acute and chronic models of seizures result in anticonvulsant effects, may be antiepileptogenic, and may afford neuroprotection (8,10–14). Although moving from preclinical research to clinical applications requires that several concerns be addressed, these experimental findings open the possibility of developing novel therapeutic strategies for the treatment of intractable seizures with focal onset, such as temporal lobe epilepsy, and possibly for preventing symptomatic epilepsies.

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References

1. Rogawski MA, Loscher W. The neurobiology of antiepileptic drugs. *Nat Neurosci Rev* 2004;5:553–564.
2. Brooks-Kayal AR, Shumate MD, Jin H, Rikter TY, Coulter DA. Selective changes in single cell $GABA_A$ receptor subunit expression and function in temporal lobe epilepsy. *Nat Med* 1998;4:1166–1172.
3. Brooks-Kayal AR, Shumate MD, Jin H, Lin DD, Rikter TY, Holloway KL, Coulter DA. Human neuronal g-aminobutyric $acid_A$ receptors: coordinated subunit mRNA expression and functional correlates in individual granule cells. *J Neurosci* 1999;19:8312–8318.
4. Zhang G, Raol Y, Hsu FC, Coulter DA, Brooks-Kayal AR. Effects of status epilepticus on hippocamal GABAA receptors are age-dependent. *Neuroscience* 2004;125:299–303.
5. Roberts DS, Hu Y, Lund IV, Brooks-Kayal AR, Russek SJ. BDNF-induced synthesis of early growth response factor 3 (EFR3) controls the levels of type A GABA receptor alpha4 subunits in hippocampal neurons. *J Biol Chem* 2005;281:29431–29435.
6. Grimm D, Kay MA. Fron virus evolution to vector revolution: use of naturally occurring serotypes of adeno-associated viru (AAV) as novel vectors for human gene therapy. *Curr Gene Ther* 2003;3:281–304.
7. Fitzsimons HL, During MJ. Design and optimization of expression cassettes including promoter choice and regulatory elements. In: Kaplitt MG, During MJ, eds. Gene therapy in the central nervous system. From bench to bedside. Academic Press, 2006:1–16.
8. Haberman RP, Samulski RJ, McCown TJ. Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. *Nat Med* 2003;9:1076–1080.
9. Davidson BL, Stein CS, Heth JA, Martins I, Kotin RM, Derksen TA, Zabner J, Ghodsi A, Chiorini JA. Recombinant adeno-associated virus type 2, 4, and 5 vectors: transduction of variant cell types and regions in the mammalian central nervous system. *Proc Natl Acad Sci* 2000;97:3428–3432.
10. Richichi C, Lin EJ, Stefanin D, Colella D, Ravizza T, Grignaschi G, Veglianese P, Sperk G, During MJ, Vezzani A. Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. *J Neurosci* 2004;24:3051–3059.
11. Haberman R, Criswell H, Snowdy S, Ming Z, Breese G, Samulski R, McCown T. Therapeutic liabilities of in vivo viral vector tropism: adeno-associated virus vectors, NMDAR1 antisense, and focal seizure sensitivity. *Mol Ther* 2002;6:495–500.
12. Lin E-JD, Richichi C, Young D, Baer K, Vezzani A and During MJ. Recombinant AAV-mediated expression of galanin in rat hippocampus suppresses seizure development. *Eur J Neurosci* 2003;18:2087–2092.
13. Noé F, Nissinene J, Pitkanen A, Gobbi M, Sperk G, During MJ, Vezzani A. Gene therapy in epilepsy: the focus on NPY. *Peptides* 2006;28:377–383.
14. Noé F, Nissinen J, Filippi F, During MJ, Pitkanen A, Vezzani A. rAAV-mediated NPY gene expression in the hippocampus of chronically epileptic rats reduces spontaneous seizures ensuing after electrically-induced status epilepticus. Annual Meeting AES, Washington, DC, December 2–5 2005. *Epilepsia* 2005;46(Suppl 8):120.