CURRENT LITERATURE IN BASIC SCIENCE

(WHAT TO DO) WHEN EPILEPSY GENE MUTATIONS STOP MAKING SENSE

An Epilepsy Mutation in the Sodium Channel *SCN1A* That Decreases Channel Excitability. Barela AJ, Waddy SP, Lickfett JG, Hunter J, Anido A, Helmers SL, Goldin AL, Escayg A. *J Neurosci* 2006;26:2714–2723. Mutations in three voltage-gated sodium channel genes *SCN1A*, *SCN2A*, and *SCN1B* and two $GABA_A$ receptor subunit genes *GABRG2* and *GABRD* have been identified in families with generalized epilepsy with febrile seizures plus ($GEFS^+$). A novel mutation, R859C, in the $Na_v1.1$ sodium channel was identified in a four-generation, 33-member white family with a clinical presentation consistent with $GEFS^{++}$. The mutation neutralizes a positively charged arginine in the domain 2 S4 voltage sensor of the $Na_v1.1$ channel subunit. This residue is conserved in mammalian sodium channels as well as in sodium channels from lower organisms. When the mutation was placed in the rat $Na_v1.1$ channel and expressed in *Xenopus* oocytes, the mutant channel displayed a positive shift in the voltage dependence of sodium channel activation, slower recovery from slow inactivation, and lower levels of current compared with the wild-type channel. Computational analysis suggests that neurons expressing the mutant channel have higher thresholds for firing a single action potential and for firing multiple action potentials, along with decreased repetitive firing. Therefore, this mutation should lead to decreased neuronal excitability, in contrast to most previous $GEFS^+$ sodium channel mutations, which have changes predicted to increase neuronal firing.

Sodium Channel Dysfunction in Intractable Childhood Epilepsy with Generalized Tonic–Clonic Seizures Rhodes TH, Vanoye CG, Ohmori I, Ogiwara I, Yamakawa K, George AL Jr. *J Physiol* 2005;569(Pt 2):433–445. Mutations in *SCN1A*, the gene encoding the brain voltage-gated sodium $channel_1$ subunit ($Na_V1.1$), are associated with genetic forms of epilepsy, including generalized epilepsy with febrile seizures plus ($GEFS^+$ type 2), severe myoclonic epilepsy of infancy (SMEI), and related conditions. Several missense *SCN1A* mutations have been identified in probands affected by the syndrome of intractable childhood epilepsy with generalized tonic–clonic seizures (ICEGTC), which bears similarity to SMEI. To test whether ICEGTC arises from molecular mechanisms similar to those involved in SMEI, we characterized eight ICEGTC missense mutations by whole-cell patch clamp recording of recombinant human SCN1A heterologously expressed in cultured mammalian cells. Two mutations (G979R and T1709I) were nonfunctional. The remaining alleles (T808S, V983A, N1011I, V1611F, P1632S, and F1808L) exhibited measurable sodium current, but had heterogeneous biophysical phenotypes. Mutant channels exhibited lower (V983A, N1011I, and F1808L), greater (T808S), or similar (V1611F and P1632S) peak sodium current densities compared with wild-type (WT)-SCN1A. Three mutations (V1611F, P1632S, and F1808L) displayed hyperpolarized conductance–voltage relationships, while V983A exhibited a strong depolarizing shift in the voltage dependence of activation. All mutants except T808S had hyperpolarized shifts in the voltage dependence of steady-state channel availability. Three mutants (V1611F, P1632S, and F1808L) exhibited persistent sodium current ranging from 1–3% of peak current amplitude that was significantly greater than WT-SCN1A. Several mutants had impaired slow inactivation, with V983A showing the most prominent effect. Finally, all of the functional alleles exhibited reduced use-dependent channel inhibition. In summary, *SCN1A* mutations associated with ICEGTC result in a wide spectrum of biophysical defects, including mild-to-moderate gating impairments, shifted voltage dependence, and reduced use dependence. The constellation of biophysical abnormalities for some mutants is distinct from those previously observed for $GEFS^+$ and SMEI, suggesting possible, but complex, genotype–phenotype correlations.

Single-Channel Properties of Human Nav1.1 and Mechanism of Channel Dysfunction in *SCN1A*-Associated Epilepsy. Vanoye CG, Lossin C, Rhodes TH, George AL Jr. *J Gen Physiol* 2006;127:1–14. Mutations in genes encoding neuronal voltage-gated sodium channel subunits have been linked to inherited forms of epilepsy. The majority of mutations (>100) associated with generalized epilepsy with febrile seizures plus ($GEFS^+$) and severe myoclonic epilepsy of infancy (SMEI) occur in *SCN1A* encoding the $Na_v1.1$ neuronal sodium channel subunit. Previous studies demonstrated functional heterogeneity among mutant *SCN1A* channels, revealing a complex relationship between clinical and biophysical phenotypes. To further understand the mechanisms responsible for mutant *SCN1A* behavior, we performed a comprehensive analysis of the single-channel properties of heterologously expressed recombinant WT-*SCN1A* channels. Based on these data, we then determined the mechanisms for dysfunction of two $GEFS^+$-associated mutations (R1648H, R1657C) both affecting the S4 segment of domain 4. WT-*SCN1A* has a slope conductance

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(17 pS) similar to channels found in native mammalian neurons. The mean open time is 0.3 ms in the –30 to –10 mV range. The R1648H mutant, previously shown to display persistent sodium current in whole-cell recordings, exhibited similar slope conductance but had an increased probability of late reopening and a subfraction of channels with prolonged open times. We did not observe bursting behavior and found no evidence for a gating mode shift to explain the increased persistent current caused by R1648H. Cells expressing R1657C exhibited conductance, open probability, mean open time, and latency to first opening similar to WT channels but reduced whole-cell current density, suggesting decreased number of functional channels at the plasma membrane. In summary, our findings define single-channel properties for WT-*SCN1A*, detail the functional phenotypes for two human epilepsy-associated sodium channel mutants, and clarify the mechanism for increased persistent sodium current induced by the R1648H allele.

COMMENTARY

Many topics are rendered less intriguing by involuntary, early exposure. It is possible that for many neuroscientists the sodium channel could be included in this category. Qualitative treatments of Hodgkin and Huxley analysis of the sodium currents underlying the action potential in squid giant axon are found in introductory texts and universally taught, just following explanations of the Nernst equation and the resting membrane potential (1). Although useful for introducing many essential concepts, this early positioning forces shortcuts and simplifications. Information on the s4 positive charge-bearing helical voltage sensor, voltage-dependent opening (activation), and closing (deactivation) is taught, as is how the channels rapidly enter an inactivated state that prevents reopening. Indeed, this process of inactivation and the associated refractory period ensures the unidirectional flow of the nerve impulse. Pedagogy that emphasizes the reliable, uniform, all-or-none aspects of sodium channel function during nondecremental propagation of action potentials leaves them seeming a little bland. The new studies reviewed here describe sodium channels that behave in unexpected and perplexing ways. Although confusing, these findings hold the potential for provoking broader interest in these seemingly well-known channels.

Over the past 15 years, mutations in genes encoding several skeletal muscle, cardiac, and nerve sodium channel subunits have been identified in patients with disorders characterized by paroxysmal hyperexcitability, including forms of periodic paralysis, hereditary ventricular arrhythmia, and epilepsy (2). Electrophysiological analysis in heterologous cells has revealed that many of the disease-provoking mutant channels increase channel openings, sometimes by enhanced activation but most commonly by causing abnormally delayed and/or incomplete inactivation. These observations fit with the view that sodium channels in mammalian excitable cells, as in squid axon, must function in a uniform way and that even slight excesses above the normal activity level could lead to symptomatic hyperexcitability (3).

As the number of known epilepsy-linked sodium channel mutations has grown and the spectrum of associated syndromes has broadened (see Stafstrom Basic Review in this issue), it has become clear that the model of how channel dysfunction can lead to epilepsy is oversimplified. Alekov et al. showed that an epilepsy mutation could be associated with enhanced inactivation predicted to result in a decrease in sodium channel currents (4). Most dramatically, a large number of frame-shift mutations expected to result in truncated, nonconducting channel proteins have been found in cases of severe myoclonic epilepsy of infancy (SMEI) (2). How can mutations that increase and decrease the activity in the same channel lead to epilepsy syndromes of variable but overlapping severity, from mild (simple febrile seizures) to catastrophic (SMEI)?

The current papers highlight this nettlesome issue through rigorous biophysical study of additional mutations in *SCN1A*, encoding the channel subunit $Na_v1.1$. The R859C mutant channels described by Barela and Waddy et al. require greater membrane depolarization for activation than the wild type, a change predicted to reduce currents in vivo. The eight mutants analyzed by Rhodes et al. include two that fail to form functional channels; the others exhibit quite heterogeneous changes in properties. Vanoye et al. use elegant single-channel recordings to define the kinetic changes underlying the behavior of two mutations involving neighboring residues on the same transmembrane segment of $Na_v1.1$. Consistent with previous whole-cell patch-clamp studies of the mutations, one increases openings, reflecting a defect in inactivation gating, while the other shows normal opening and closing kinetics but a lowered total number of functional numbers.

Although reductive approaches of this kind are invaluable, the contribution of an ion channel to behavior can only be discerned once its functional profile is understood at several levels—molecular, subcellular, cellular, and neuronal network. Given its importance, it is surprising how little is known about the cell biology of neuronal $Na_v1.1$. Unlike in skeletal muscle and heart where a single type of sodium channel predominates, in brain, individual neurons simultaneously express multiple varieties of sodium channels (5). The classic role of initiation and propagation of action potentials in axons is mainly the responsibility of $Na_v1.6$ (and in some instances $Na_v1.2$), channels that so far only rarely have been implicated in human

epilepsy (6). $Na_v1.1$ (encoded by *SCN1A*, for which over 100 human epilepsy mutations are known) appears to be expressed at low-to-moderate densities but not typically on axons. Instead, $Na_v1.1$ appears to contribute to the excitability of neuronal somata and dendrites, helping to shape excitatory postsynaptic potentials and supporting the backpropagation of action potentials into dendrites; however, the specific in vivo functional profile of $Na_v1.1$ needs to be far better understood.

Much can by learned by combining molecular, cell biological, and electrophysiological approaches to analyze function of mutant neuronal channels in vivo. For example, one of the mysteries regarding benign familial neonatal seizures (BFNS) has been that the mutations in *KCNQ2* and *KCNQ3* potassium channel subunits, which cause the disorder, often have very little effect on channel function when expressed in cell lines or *Xenopus* oocytes (7). New work suggests that these channels have previously unsuspected roles on axons and that some of the BFNS mutants are transported quite inefficiently to their proper axonal targets (8,9). A way in which a sodium channel loss-of-function mutation could lead to hyperexcitability in a neuronal circuit is illuminated by other recent studies involving $Na_v1.1$ knockout mice (10). Hippocampal inhibitory neurons from the mutants (but not excitatory pyramidal cells) show a dramatic reduction in detectable sodium channel current, suggesting that seizures in these mice could result from a loss of inhibition in cortical circuits. The inhibitory neurons also show a remarkable compensatory increase in expression of $Na_v1.3$, a sodium channel isoform with biophysical properties quite different from the missing $Na_v1.1$ channels. Further analysis of these mutant mice and of mice bearing missense mutations associated with human epilepsy may answer the unsettled questions about $Na_v1.1$ raised by the current papers. Along the way, investigators will likely have to discard the simplified notion that the function of sodium channels in the neurons is restricted to faithfully "reporting out" the decisions made by synapses—instead, these channels may well be found in the thick of the action.

by Edward C. Cooper, MD, PhD

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Respiratory Alkalosis: "Basic" Mechanism of Febrile Seizures?

Experimental Febrile Seizures Are Precipitated by a Hyperthermia-Induced Respiratory Alkalosis. Schuchmann S, Schmitz D, Rivera C, Vanhatalo S, Salmen B, Mackie K, Sipila ST, Voipio J, Kaila K. *Nat Med* 2006;12:817–823. Febrile seizures are frequent during early childhood, and prolonged (complex) febrile seizures are associated with an increased susceptibility to temporal lobe epilepsy. The pathophysiological consequences of febrile seizures have been extensively studied in rat pups exposed to hyperthermia. The mechanisms that trigger these seizures are unknown, however. A rise in brain pH is known to enhance neuronal excitability. Here we show that hyperthermia causes respiratory alkalosis in the immature brain, with a threshold of 0.2–0.3 pH units for seizure induction. Suppressing alkalosis with 5% ambient CO_2 abolished seizures within 20 s. CO_2 also prevented two long-term effects of hyperthermic seizures in the hippocampus: the upregulation of the I_h current and the upregulation of CB1 receptor expression. The effects of hyperthermia were closely mimicked by intraperitoneal injection of bicarbonate. Our work indicates a mechanism for triggering hyperthermic seizures and suggests new strategies in the research and therapy of fever-related epileptic syndromes.

Epilepsy Currents, Vol. 7, No. 1 (January/February) 2007 pp. 25–27
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COMMENTARY

Fever represents a typical response to infection at all ages. However, only in young children is prolonged fever capable of inducing convulsions. While febrile seizures are generally considered benign, there is emerging evidence that in certain cases they can lead to chronic epilepsy. Several factors, such as altered phenotype of hyperpolarization-activated cyclic nucleotide-gated channels, altered hyperpolarization-activated cation current (I_h), enhanced endocannabinoid signaling, and mossy fiber sprouting, have been implicated in the mechanisms of increased neuronal excitability and of epilepsy following febrile seizures (1–3). However, in order to prevent long-term pathophysiological sequela, it is important to understand the basic mechanisms that trigger febrile seizures per se and why febrile seizures usually only occur in pediatric population.

Because fever is commonly associated with inflammation, inflammatory cytokines have been regarded as candidate mechanistic factors of febrile convulsions. However, data accumulated to date do not provide compelling evidence that inflammatory cytokines are directly involved in the development of febrile seizures. Thus, while some reports link interleukin-1β (IL-1β) gene polymorphisms with febrile seizures (4), other studies did not find any association between the two phenomena (5). A study using IL-1β receptor deficient mice proved that IL-1β, a key inflammatory cytokine, was not required for febrile seizures to occur, although it could have a modulatory effect (6). Furthermore, inflammatory cytokines are expressed both in adult and in immature brain and have been shown to regulate adult epileptogenesis (7); thus, inflammatory cytokines alone cannot explain age specificity or, consequently, the mechanisms of febrile convulsions.

Two features of febrile seizures—rapid onset and age selectivity—suggest that certain highly reactive mechanisms specific for the immature age are responsible for their occurrence and progression. Schuchmann and colleagues focused their study on the examination of these mechanisms. The authors exploited the well-established fact that fever is commonly accompanied by compensatory hyperventilation, which in turn might lead to an alkaline shift in pH as a result of a decrease in the partial pressure of CO_2. At the same time, elevated brain pH is known to enhance neuronal excitability.

Schuchmann and coworkers performed a series of elegant experiments designed to connect the dots and identify mechanisms that may underlie the occurrence of febrile seizures. They compared behavioral, electrographic, physiologic, and chemical responses to hyperthermia induced in immature rats of two ages: 8–11 days, when seizures readily develop in response to the elevating of core temperature, and 3 weeks, when the increase in body temperature does not result in seizure responses. Their major findings were that (a) hyperthermia led to a 60% increase in breathing rate in younger rats, whereas in 3-week-old animals, a similar rise in body temperature increased breathing rate by 28%; (b) in younger animals, hyperventilation was accompanied by a 3% increase in brain pH, while at 3 weeks pH increase was 0.5%; (c) as expected, younger but not older rats developed seizures in response to hyperthermia. These observations were followed by simple, yet impressive, experiments. The authors showed that by directly elevating pH to the same level as induced by hyperthermia (using systemic injection of bicarbonate), behavioral and EEG seizures could be readily induced in 8- to 11-day-old rats. However, they did not examine whether a similar injection of bicarbonate to 3-week-old animals would have failed to induce alkalinization and seizures; such an experiment would have further validated their hypothesis.

One logical conclusion and practical implication of the findings of Schuchmann and coworkers is that normalizing partial pressure of CO_2 may be effective in blocking febrile convulsions in rat pups. Indeed, the authors found that the application of 5% CO_2 to the inhaled air completely blocked electrographic and behavioral manifestations of febrile convulsions. Even more remarkably, long-term consequences of febrile seizures, such as upregulation of I_h current and overexpression of cannabinoid receptors, were prevented by the CO_2 therapy. Although the authors did not explore whether such treatment also blocked long-term enhanced excitability and predisposition to seizures (which would make their findings even more exciting), they showed that apparent substrates of postfebrile seizure-induced epileptogenesis were blocked.

The question of why younger animals, compared to older animals, developed more profound hyperventilation that was sufficient to raise pH to the seizure-inducing level was not directly addressed in the experiments. However, it has been established that the lowest ontogenic chemosensitivity to CO_2 occurs in rats around postnatal day 10, which is precisely when febrile seizures occur. Hence, central feedback mechanisms that control respiratory rate based on the partial pressure of CO_2 are not mature in younger animals. The inability to keep CO_2 concentration within physiological parameters might eventually lead to tissue alkalinization and ultimately to seizures.

The importance of these studies also might extend beyond an understanding of the mechanisms of febrile seizures. While respiratory alkalosis appears to be age- and model specific, it is quite possible that shifts in brain pH, in general, could play an important role at various ages and in other types of epilepsy. Accordingly, it has been shown that a focal increase in pH in chronic epileptic adult animals is associated with the generation of spontaneous interictal spikes and could contribute to the interictal–ictal transition (8). Therefore, when superimposed on chronically modified neuronal circuits in the epileptic

brain, the momentary alkalinization that occurs as a result of normal variations in pH might be a mechanism by which individual seizures are triggered in epileptic patients. In this regard, hyperventilation is long known to induce interictal spikes and is commonly used for the EEG diagnosis of epilepsy. Furthermore, carbonic anhydrase inhibitors, such as acetazolamide, are known to exert anticonvulsant effects (9). Clearly, the findings of Schuchmann and colleagues offer important basic and translational implications. If proven true in the clinical environment, these data could provide a simple, safe, and effective treatment for febrile seizures in infants, with both immediate and long-term benefits.

by Andrey M. Mazarati, MD, PhD

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The Dual Roles of GABA in Seizures and Epilepsy Generate More Excitement

Anomalous Levels of Cl– Transporters in the Hippocampal Subiculum from Temporal Lobe Epilepsy Patients Make GABA Excitatory. Palma E, Amici M, Sobrero F, Spinelli G, Di Angelantonio S, Ragozzino D, Mascia A, Scoppetta C, Esposito V, Miledi R, Eusebi F. *Proc Natl Acad Sci USA* 2006;103:8465–8468. Erratum in *Proc Natl Acad Sci USA* 2006;103:11814. The mRNA levels of NKCC1, an inwardly directed Na^+, K^+-$2Cl^-$ cotransporter that facilitates the accumulation of intracellular Cl^-, and of KCC2, an outwardly directed K^+-Cl^- cotransporter that extrudes Cl^-, were studied in surgically resected brain specimens from drug-resistant temporal lobe (TL) epilepsy (TLE) patients. Quantitative reverse transcription polymerase chain reaction (RT-PCR) analyses of the mRNAs extracted from the human TLE-associated brain regions revealed an upregulation of NKCC1 mRNA and a downregulation of KCC2 mRNA in the hippocampal subiculum, compared with the hippocampus proper or the TL neocortex, suggesting an abnormal transcription of Cl^- transporters in the TLE subiculum. In parallel experiments, cell membranes isolated from the same TLE-associated brain regions were injected into *Xenopus* oocytes that rapidly incorporated human $GABA_A$ receptors into their surface membrane. The GABA currents elicited in oocytes injected with membranes from the subiculum had a more depolarized reversal potential (E_{GABA}) compared with the hippocampus proper or the neocortex. The NKCC1 blocker bumetanide or a temperature decrease of 10°C shifted the GABA-current E_{GABA} more negative in oocytes injected with membranes from TLE hippocampal subiculum, matching the E_{GABA} of TL neocortex-injected oocytes. We conclude that the anomalous expression of both Cl^- transporters, KCC1 and NKCC2, in TLE hippocampal subiculum probably causes altered Cl^- transport in the "epileptic" neurons, as revealed in the microtransplanted *Xenopus* oocytes, and renders GABA aberrantly "exciting," a feature that may contribute to the precipitation of epileptic seizures. The authors note that the last sentence of the abstract should read: "We conclude that the anomalous expression of both Cl^- transporters, NKCC1 and KCC2, in TLE hippocampal subiculum probably causes altered Cl^- transport in the 'epileptic' neurons, as revealed in the microtransplanted *Xenopus* oocytes, and renders GABA aberrantly "exciting," a feature that may contribute to the precipitation of epileptic seizures. This error does not affect the conclusions of the article.

Epileptogenic Actions of GABA and Fast Oscillations in the Developing Hippocampus. Khalilov I, Le Van Quyen M, Gozlan H, Ben-Ari Y. *Neuron* 2005;48:787–796. GABA excites immature neurons and inhibits adult ones, but whether this contributes to seizures in the developing brain is not known. We now report that in the developing, but not the adult, hippocampus, seizures beget seizures only if GABAergic synapses are functional. In the immature hippocampus, seizures generated with functional GABAergic synapses include fast oscillations that are required to transform a naive network to an epileptic one: blocking GABA receptors prevents the long-lasting sequels of seizures. In contrast, in adult neurons, full blockade of GABA(A) receptors generates epileptogenic high-frequency seizures. Therefore, purely glutamatergic seizures are not epileptogenic in the developing hippocampus. We suggest that the density of glutamatergic synapses is not sufficient for epileptogenesis in immature neurons; excitatory GABAergic synapses are required for that purpose. We suggest that the synergistic actions of GABA and NMDA receptors trigger the cascades involved in epileptogenesis in the developing hippocampus.

COMMENTARY

A dramatic rethinking of the role of GABA—traditionally regarded as the brain's main inhibitory neurotransmitter—in seizures and epilepsy has been occurring in recent years. The shift in thinking began more than 10 years ago with the discoveries that during the early stages of brain development, GABA acts as an excitatory neurotransmitter and plays a key role in the shaping of synaptic connections. Because GABA exerts its primary (fast) effects through chloride currents associated with $GABA_A$ receptors, it either depolarizes or hyperpolarizes the postsynaptic neuron, depending on chloride's electrochemical gradient across the neuronal membrane. Early in development, expression of the $Na^+/K^+/Cl^-$-cotransporter, NKCC1, results in a high intracellular chloride concentration that makes GABA depolarizing: negatively charged chloride ions flow out of the cell upon $GABA_A$ receptor activation. During the first two postnatal weeks in rats and likely during the late prenatal and early postnatal period in humans, the chloride concentration within neurons decreases simultaneously with a decrease in expression of NKCC1 and an increase in expression of the K^+/Cl^--cotransporter, KCC2. KCC2 transports chloride out of the neuron, resulting in the low intracellular chloride concentration found in mature neurons that makes GABA hyperpolarizing.

Models of brain injury in adult animals, including prolonged epileptiform activity, have demonstrated a downregulation of neuronal KCC2 expression and a transformation of GABA into a depolarizing, excitatory neurotransmitter in affected brain regions (1). Therefore, it is possible that acute and chronic changes in chloride transporter expression, by reducing

Epilepsy Currents, Vol. 7, No. 1 (January/February) 2007 pp. 28–30
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or even reversing the inhibitory influence of GABA, underlie the generation of seizures acutely or the process of epileptogenesis. If this were true, pharmacological agents that act at chloride transporters could provide novel anticonvulsant therapies. Indeed, the loop diuretic bumetanide, which potently and selectively blocks NKCC1, reduces chloride accumulation in neurons and shifts the reversal potential for GABA toward more hyperpolarized levels, thereby reducing or even terminating seizure activity in the immature mouse brain (2,3).

Another aspect of the changing perspective on GABA concerns the key role it plays in the development of neuronal circuits. It is a major excitatory neurotransmitter in the immature brain at a time when glutamatergic synaptic connections are beginning to mature (4). In the early postnatal period in rats, the pairing of depolarizing GABA-mediated responses with glutamate release at immature excitatory synapses that contain only *N*-methyl-D-aspartate (NMDA) receptors relieves their voltage-dependent block by magnesium ions. The resulting NMDA receptor-mediated calcium influx provides the signal required for insertion of AMPA-type glutamate receptors into the postsynaptic membrane and, therefore, leads to their maturation into active synapses. Given this crucial role in the development of excitatory synapses, it is likely that the depolarizing action of GABA contributes to the particular vulnerability of neonates to seizures and epilepsy. This developmental process appears to be analogous to the insertion of AMPA receptors that occurs during induction of long-term potentiation in more mature brain circuits, when glutamate provides the depolarizing signal. Considering the importance of long-term potentiation in learning and memory functions, disruption of these analogous signaling patterns by seizures may have long-lasting consequences in patients at a vulnerable age.

Several questions have been raised by these recent findings. First, do changes in chloride transporter expression that recapitulate early development occur in adult epilepsy, such that GABA has less inhibitory efficacy or perhaps is excitatory? Second, because GABA is a major excitatory neurotransmitter early in development, what role does it play in the generation of seizures and in the development of chronic epilepsy? Two new studies have begun to address these questions.

Although it is now clear that the high expression of NKCC1 in immature brain does play a role in the increased susceptibility of neonates to seizures, whether altered chloride transporter expression plays a role in adult human epilepsy remains unclear. To explore this possibility, Palma et al. measured the expression of NKCC1 and KCC2 in temporal lobe resected from four patients with hippocampal sclerosis who underwent surgery for the treatment of intractable epilepsy. Comparing mRNA levels in the temporal neocortex, hippocampus, and subiculum, they found that on average NKCC1 was upregulated in the subiculum approximately threefold compared with the cortex. In addition, KCC2 was downregulated by approximately 80% in the subiculum. Although not part of the hippocampus proper, the subiculum is the major output region from the hippocampus, receiving inputs from CA1 pyramidal neurons and sending outputs to many other brain regions. Interestingly, one report of electrophysiological recordings from acute slices of resected human temporal lobe found the subiculum to be the origin of interictal-like epileptiform activity (5), suggesting that it may be an important locus of epileptogenesis. The mRNA changes, if they are reflected in altered protein levels, are expected to shift neuronal chloride gradients and make GABA less hyperpolarizing. The authors investigated the functional consequences of this expression pattern by injecting membranes prepared from the subiculum, hippocampus, and cortex into *Xenopus* oocytes. This technique effectively reconstituted in oocyte membranes the complement of GABA receptors and chloride transporter proteins expressed in those regions. When membranes from subiculum were used, the reversal potential for chloride, as determined by application of GABA, was shifted toward more depolarized potentials compared with those of hippocampus or cortex. In support of the hypothesis that an increased ratio of NKCC1 to KCC2 is responsible for this difference, application of bumetanide shifted the chloride reversal potential from subicular membranes toward the values in hippocampus and cortex. These findings suggest that chloride transporter expression in adult epileptic subiculum is similar to early stages of development. The results of Palma and colleagues suggest that in the epileptic subiculum, GABA has diminished efficacy as an inhibitory transmitter.

Upregulation of NKCC1, and consequently reduced inhibition, in chronic epilepsy may be one determinant of the intractability of seizures to standard drug treatments. Of course, this study only examined tissue from four patients with intractable epilepsy, so it remains to be seen whether the finding can be generalized to all intractable patients. Moreover, because no nonepileptic brains were examined and the authors used the same patients' temporal neocortex as a "control," it is not clear whether this pattern of transporter expression is truly abnormal. If these expression patterns are related to epilepsy, when did the changes occur? It is possible that they occurred before the onset of clinical epilepsy and were part of the process of epileptogenesis or that they were the result of years of seizures that were ineffectively controlled. It also is possible that the changes were present early in the disease and were part of the reason that medications were not effective. Studies involving tissue from human patients present many challenges, but the findings in this study and the questions they raise should stimulate further research using animal models to address the relationship between chloride transporter expression, epileptogenesis, and the response of seizures to treatment.

Another recent study examined the role of GABA in the generation of seizures and chronic epilepsy in immature and mature hippocampus. Khalilov et al. used a preparation composed

of both hippocampi from an immature rat and the commissural fibers that connect them, which enabled the investigators to perfuse each hippocampus separately. They had demonstrated earlier (6) that seizures induced in one hippocampus propagate to the contralateral side acutely and after repeated seizures, produce lasting epileptogenic changes in the contralateral hippocampus (i.e., a "mirror focus"). The current study extended those findings to explore the mechanisms involved in the transition from acute, induced seizures to chronic, spontaneous seizures, mimicking the development of epilepsy. When inhibition was blocked (with GABA antagonists) or excitation enhanced (with kainic acid) in the ipsilateral hippocampus, acute seizure-like discharges were generated both in the ipsilateral and contralateral hippocampi. Repeated induction of ipsilateral seizures with either treatment was epileptogenic to the contralateral side, resulting in spontaneously generated seizures—even after the commissural connection was cut. But only repeated kainate treatment was epileptogenic on the ipsilateral side. Blocking GABA receptors appeared to protect the ipsilateral hippocampus from undergoing the long-lasting changes associated with chronic epilepsy, supporting the view that the action of GABA itself is required to produce the synaptic changes that underlie epilepsy in the immature brain.

Khalilov and colleagues also examined characteristics of the seizure patterns induced by both GABA antagonists and kainate; they found that during the ipsilateral kainate-induced seizures as well as during the propagated, contralateral seizures, fast oscillations were observed during the ictal discharges. Fast oscillations are rapid network-driven discharges (40–140 Hz) that depend in part on GABAergic interneurons, so seizures induced by GABA antagonists do not exhibit these discharges. The authors found that these largely interneuron-driven patterns of activity, while not required to generate seizures, are required to produce the changes associated with epilepsy. Both the fast oscillations and the epileptogenesis were prevented by an NMDA antagonist, suggesting that both GABA and NMDA receptors act together to promote these network patterns of activity and the lasting changes that result from them. Therefore, it appears that there is a critical developmental period during which GABAergic networks are mature enough to produce fast oscillations and, when driven in conjunction with NDMA receptor-containing synapses, can produce persistent epileptic changes.

Using adult rat hippocampi (i.e., after GABA becomes inhibitory and the critical developmental periods are over), Khalilov et al. assessed whether requirements for GABAergic synaptic activity and fast oscillations for epileptogenesis were similar to those of the immature brain. Unable to use the same intact preparation they used in immature animals, they placed adult and immature hippocampi in the same chamber. In contrast to the immature brain in which only kainate produced lasting epileptic changes, both GABA antagonists and kainate were epileptogenic in adult hippocampus. These results indicate that GABAergic synapses are only required for epileptogenesis in immature brain. Their results point to a special role of GABA throughout a critical period of development, corresponding to the neonatal period in humans. During this time, GABAergic synapses are capable of driving ictal discharges as well as producing, in conjunction with NMDA-containing synapses, the chronic changes associated with epilepsy.

GABA's actions in the developing and mature brain are much more complex than suggested by earlier attempts to classify it simply as an inhibitory neurotransmitter. Because of the effects of GABA on developing synaptic connections, it is likely that seizures in the immature brain disrupt normal developmental processes and possibly produce long-lasting alterations in brain circuitry that either predispose to future seizures or to other neurological problems. Accordingly, the use of GABAergic modulators, such as benzodiazepines and barbiturates, to prevent seizures may not only lack the efficacy in neonates that is evidenced in adults, but also may interfere with normal developmental processes—at least theoretically. The impact of these considerations on clinical decision making will not be clear until more studies are available that examine the effects of both seizures and their treatment on developmental processes. Finally, the role of GABA in adult epilepsy is being reexamined in light of increasing evidence that altered chloride transporter expression may underlie the aberrant excitability within the epileptic tissue or may be a determinant of drug treatment response. These findings may lead to the development of novel therapeutics aimed at normalizing chloride distribution in brain regions with abnormal chloride transporter expression.

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