SEIZURES BEGET SEIZURES: A LACK OF EXPERIMENTAL EVIDENCE AND CLINICAL RELEVANCE FAILS TO DAMPEN ENTHUSIASM

Three Brief Epileptic Seizures Reduce Inhibitory Synaptic Currents, $GABA_A$ Currents, and $GABA_A$-Receptor Subunits. Evans MS, Cady CJ, Disney KE, Yang L, LaGuardia JJ. *Epilepsia* 2006;4710):1655–1664. PURPOSE: Cellular mechanisms activated during seizures may exacerbate epilepsy. γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in brain, and we hypothesized that brief epileptic seizures may reduce GABA function. METHODS: We used audiogenic seizures (AGSs) in genetically epilepsy-prone rats (GEPRs) to investigate effects of seizures on GABA-mediated inhibition in the presence of epilepsy. GEPRs are uniformly susceptible to AGSs beginning at 21 postnatal days. AGSs are brief convulsions lasting 20 s, and they begin in inferior colliculus (IC). We evoked three seizures in GEPRs and compared the results with those in seizure-naive GEPRs and nonepileptic Sprague-Dawley (SD) rats, the GEPR parent strain. RESULTS: Whole-cell recording in IC slices showed that GABA-mediated monosynaptic inhibitory postsynaptic currents (IPSCs) were reduced 55% by three brief epileptic seizures. Whole-cell recording in IC neuronal cultures showed that currents elicited by GABA were reduced 67% by three seizures. Western blotting for the alpha1 and alpha4 subunits of the $GABA_A$ receptor showed no statistically significant effects. In contrast, three brief epileptic seizures reduced gamma2 subunit levels by 80%. CONCLUSIONS: The effects of the very first seizures, in animals known to be epileptic, in an area of brain known to be critical to the seizure network, were studied. The results indicate that even brief epileptic seizures can markedly reduce IPSCs and GABA currents and alter $GABA_A$-receptor subunit protein levels. The cause of the reductions in IPSCs and GABA currents is likely to be altered receptor subunit composition, with reduced gamma2 levels causing reduced $GABA_A$-receptor sensitivity to GABA. Seizure-induced reductions in GABA-mediated inhibition could exacerbate epilepsy.

COMMENTARY

Whether "seizures beget seizures" has been a point of contention ever since Sir William Gowers coined this aphorism more than 125 years ago (1). Although there is convincing experimental evidence to support this premise, current understanding suggests that it is not clinically applicable and that, with the exception of some rare syndromes, human epilepsy is not a progressive, self-perpetuating disorder (2). Potentially clouding this knowledge is the increasing recognition that early life episodes of complex febrile seizures are associated with the later development of temporal lobe epilepsy (3) and that the number of pretreatment seizures is related to the probability of subsequent remission (4). These findings are, of course, entirely separate issues from the suggestion that one seizure increases the likelihood of another.

Unfortunately, the boundaries of these phenomena have become somewhat blurred amid the recent clamor to investigate the cellular mechanisms of epileptogenesis and to assess how these mechanisms might be exploited to prevent or delay the development of epilepsy (5). The procedure of employing acute experimental seizures as a precipitant of a subsequent epileptic state and dissecting the myriad of molecular events that occur in the latent period is a perfectly reasonable and legitimate endeavor. However, a troubling departure from this effort has involved a regression to Gowers's dictum and resulted in a largely unwritten acceptance of the theory that a single seizure or cluster of seizures can predispose to further episodes. In their enthusiasm preclinical investigators can, on occasion, lose sight of the importance of clinical relevance and, more specifically, the fact that epileptogenesis, pharmacoresistance, and seizures begetting seizures are not one and the same thing.

The recent manuscript by Evans et al. examined the effect of three successive audiogenic seizures on the GABA neurotransmitter system in the inferior colliculus of the genetically epilepsy-prone rat (GEPR). Twenty-four hours after the final seizure, the investigators observed a pattern of cellular effects that was consistent with an alteration in the subunit composition of the postsynaptic $GABA_A$ receptor, leading to a decrease in its sensitivity to GABA and an attenuation of inhibitory neurotransmission in the site of seizure origin. They deduced that compromised GABAergic inhibition in the inferior colliculus could predispose to further seizures and contribute to the phenomenon of audiogenic kindling (6–8). This has, after all, been mooted as one of the principal mechanisms of seizure susceptibility in the GEPR (9). However, the authors chose

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not to comment on the apparent hyperactivity of GABAergic inhibition in the inferior colliculus of seizure-naive, epilepsy-prone rats, when compared to nonepileptic control animals. Arguably, this is a more intriguing finding—one that may underlie the epileptogenic nature of the aforementioned diminution in GABAergic activity, and one that certainly has a significant bearing on how this study could or should be interpreted. Instead, the authors elected to focus on clinical relevance, suggesting that their study might explain the phenomenon of seizure clustering and have implications for epileptogenesis, pharmacological responsiveness, and the treatment of epilepsy after a single unprovoked seizure.

At this stage, the margins of disparate clinical issues begin to merge and interpretation becomes a little questionable. On the surface, these investigators have succeeded in identifying a mechanism by which seizures *might* beget seizures, at least in the GEPR. However, it is not appropriate to then extrapolate this observation to the clinical arena where the phenomenon does not exist or attempt to align it with any other vaguely related clinical circumstance. There is no doubt that the study provides a novel insight into the cellular consequences of audiogenic stimulation in the GEPR, but it also offers up more questions than answers. The permanence of the observed effects and how they relate to the number and/or frequency of seizures is not addressed, and the authors fail to discount the possibility that repeated exposure to intense audiogenic provocation might elicit similar changes in the inferior colliculus of normal animals, particularly as this structure represents the primary point of convergence for multiple, bilateral auditory afferents (10). Finally, they provide no direct experimental evidence that would support their proposed exacerbation of seizures. Demonstrating that seizure severity increased with successive stimulations would have added a behavioral correlate to the cellular and molecular findings and offered at least some support to the principal findings of this manuscript.

Despite the authors' assertions to the contrary, there is little in their paper to confirm that repeated seizures are associated with enhanced epileptogenicity in the GEPR and nothing to suggest that these findings have any relevance to the exacerbation of clinical epilepsy. This investigation has elegantly demonstrated the effect of a single seizure or a brief cluster of seizures on GABA-mediated inhibition in the primary epileptogenic zone in the GEPR but any interpretation of the findings should end there. In one sense, the conclusions of this paper are a little misguided, possibly as a result of ongoing efforts to unravel the phenomena of epileptogenesis, pharmacoresistance, and self-perpetuating seizures. In another sense, however, they are in keeping with an increasing extravagance in contemporary scientific reporting. Seizures may not beget seizures but research trends can, on occasion, beget overinterpretation of results.

by Graeme J. Sills, PhD

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Does Leakage of the Blood–Brain Barrier Mediate Epileptogenesis?

Blood-Brain Barrier Leakage May Lead to Progression of Temporal Lobe Epilepsy. van Vliet EA, da Costa Araujo S, Redeker S, van Schaik R, Aronica E, Gorter JA. *Brain* 2007;130(Pt 2):521–534. Leakage of the blood–brain barrier (BBB) is associated with various neurological disorders, including temporal lobe epilepsy (TLE). However, it is not known whether alterations of the BBB occur during epileptogenesis and whether this can affect progression of epilepsy. We used both human and rat epileptic brain tissue and determined BBB permeability using various tracers and albumin immunocytochemistry. In addition, we studied the possible consequences of BBB opening in the rat for the subsequent progression of TLE. Albumin extravasation in human was prominent after status epilepticus (SE) in astrocytes and neurons, and also in hippocampus of TLE patients. Similarly, albumin and tracers were found in microglia, astrocytes and neurons of the rat. The BBB was permeable in rat limbic brain regions shortly after SE, but also in the latent and chronic epileptic phase. BBB permeability was positively correlated to seizure frequency in chronic epileptic rats. Artificial opening of the BBB by mannitol in the chronic epileptic phase induced a persistent increase in the number of seizures in the majority of rats. These findings indicate that BBB leakage occurs during epileptogenesis and the chronic epileptic phase and suggest that this can contribute to the progression of epilepsy.

TGF-Beta Receptor-Mediated Albumin Uptake into Astrocytes Is Involved in Neocortical Epileptogenesis. Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tomkins O, Seiffert E, Heinemann U, Friedman A. *Brain* 2007; 130(Pt 2):535–547. It has long been recognized that insults to the cerebral cortex, such as trauma, ischaemia or infections, may result in the development of epilepsy, one of the most common neurological disorders. Human and animal studies have suggested that perturbations in neurovascular integrity and breakdown of the blood–brain barrier (BBB) lead to neuronal hypersynchronization and epileptiform activity, but the mechanisms underlying these processes are not known. In this study, we reveal a novel mechanism for epileptogenesis in the injured brain. We used focal neocortical, long-lasting BBB disruption or direct exposure to serum albumin in rats (51 and 13 animals, respectively, and 26 controls) as well as albumin exposure in brain slices *in vitro*. Most treated slices (72%, $n = 189$) displayed hypersynchronous propagating epileptiform field potentials when examined 5–49 days after treatment, but only 14% ($n = 71$) of control slices showed similar responses. We demonstrate that direct brain exposure to serum albumin is associated with albumin uptake into astrocytes, which is mediated by transforming growth factor β receptors (TGF-βRs). This uptake is followed by down regulation of inward-rectifying potassium (Kir 4.1) channels in astrocytes, resulting in reduced buffering of extracellular potassium. This, in turn, leads to activity-dependent increased accumulation of extracellular potassium, resulting in facilitated *N*-methyl-D-aspartate-receptor-mediated neuronal hyperexcitability and eventually epileptiform activity. Blocking TGF-βR *in vivo* reduces the likelihood of epileptogenesis in albumin-exposed brains to 29.3% ($n = 41$ slices, $P < 0.05$). We propose that the above-described cascade of events following common brain insults leads to brain dysfunction and eventually epilepsy and suggest TGF-βRs as a possible therapeutic target.

COMMENTARY

A flurry of recent papers confirms the growing interest in cerebrovascular research among epileptologists (1). After the early pioneering work by Quadbeck and Helmchen, who suggested that loss of blood–brain barrier (BBB) integrity may lead to a variety of CNS disorders including seizures, almost half a century has elapsed without significant advances in research on the BBB as it relates to epilepsy (2). In fact, most of the work on the BBB and epilepsy has focused on multiple drug resistance, with little acknowledgment of an etiologic role for cerebrovascular failure in seizure disorders. It is now known, at least in principle, that BBB disruption leads to acute seizures in humans and animal models (3,4). The two papers reviewed here further investigate the mechanisms (Ivens et al.) and etiology (van Vliet et al.) of BBB disruption in seizure disorders.

The work by Ivens and colleagues is a logical continuation of earlier studies that induced seizures in rats by exposing the brain surface to bile salt, which is believed to "open" the BBB (4). One of the most significant findings using this model is a persistent and dramatic ingress of extravasated serum albumin into astrocytes. The finding and its relationship to abnormal electrical activity were further investigated, and it was demonstrated that albumin loading of CNS glia is mediated by a specific receptor for TGF-β, a powerful regulator of apoptosis and the cell cycle. Interestingly, the putative downstream event of this altered signaling is one of the oldest suspects in epileptogenesis, namely, increases in extracellular K^+. Furthermore, the current hypothesis links cell cycle, gliosis, and expression of potassium channels, as was anticipated by Dini et al. (5).

The paper by van Vliet and colleagues tackles another aspect of the link between vascular and parenchymal factors in

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epileptogenesis. The authors showed that in the kainate model of epilepsy there is impairment of the BBB and that loss of cerebrovascular protection may be one factor in determining epileptogenesis. Thus, treatment with an osmotic agent commonly used to treat brain edema, leads to BBB leakage that is associated with an increased probability of ictal activity. The study employed standard intravascular staining techniques to demonstrate BBB leakage. Clinically, BBB integrity is assessed with gadolinium-enhanced MRI. In the laboratory, BBB function is commonly determined with markers that bind serum albumin or albumin itself conjugated to fluorophores, such as fluorescein isothiocyanate, or to Evans blue. Using this technique, Seiffert et al. described albumin accumulation into astrocytes. In the study by van Vliet, however, albumin accumulated equally well in neurons, confirming results by others (3,6).

van Vliet et al. did not explore the mechanism by which BBB leakage may contribute to epileptogenesis. Nevertheless, whatever the mechanisms, BBB failure triumphantly enters the crowded field of epileptogenic triggers. There are reasons for both cheers and jeers. The involvement of the BBB in epilepsy opens new therapeutic options, particularly when and if the targets are known and accessible. For example, assuming TGF-β is relevant, as proposed by Seiffert et al., it might be worthwhile to attempt to modulate the expression of this protein by antisense or small interfering RNA (siRNA) technology. Loss of BBB function commonly results from inflammatory changes associated or not associated with trauma, suggesting a link between seizures, the BBB, and inflammation (1). In addition to acute seizures, there is new evidence that inflammation may also play a role in epileptogenesis, although no antiinflammatory compounds have yet been shown to be protective (7). Chronic immunosuppression is fraught with concerns. However, short-term immunosuppression during the period of vulnerability following an epileptogenic stimulus, might find utility. Several issues and incongruities need to be resolved before a rational, BBB-based therapeutic approach is ready for clinical application. These include using comparable means to study the BBB in human subjects and animal models.

First and foremost, in the study of van Vliet et al., the treatment used to open the BBB is administered clinically to protect against seizures. In fact, at the concentrations these authors used, intravenous mannitol slightly elevates blood osmolarity and is commonly employed to decrease intracranial pressure via a simultaneous osmotic action on the kidney and the brain. At significantly higher concentrations (1.4 molar) and when applied intraarterially to the carotid or vertebral circulation, mannitol is used to open the BBB. When the latter procedure was used, acute seizures resulted (3). It is unclear at what concentration or dose the effect of mannitol changes from protective to damaging, and the mechanisms underlying this shift are still unknown.

The link between loss of BBB function and albumin accumulation in glia also needs further investigations. The hypothesis formulated regarding the specificity of albumin accumulation in astrocytes is not necessarily at odds with the fact that van Vliet et al. and others (3,6) found albumin in neurons as well. In fact, the data convincingly show that a small decrease in spatial buffering of extracellular K^+ occurred after exposure to albumin. However, the alternative hypothesis implicating an effect of albumin on potassium currents also should be considered. A direct action of albumin acting on potassium channels is made even more intriguing by the fact that the very method Ivens et al. used to induce epileptogenesis—bile salts—also inhibits potassium channel activity (8).

Ivens et al. found that a specific inwardly rectifying current was reduced by albumin, namely the inwardly rectifying potassium 4.1 (Kir 4.1) channel. Kir, and in particular Kir 4.1, are key regulators of glial functions, which in turn determine neuronal excitability and axonal conduction (9,10). The electrophysiological characterization of astrocytes from Kir 4.1 knockout mice showed that Kir 4.1 mediates most of the Kir current in astrocytes, but the fact that loss of Kir 4.1 did not significantly alter neuronal function suggests that these channels are one player among many in the coordinated process of extracellular potassium regulation. In the paper by Ivens et al., the effect of albumin on extracellular K^+ also was modest, suggesting that even in this model, alternative mechanisms to buffer extracellular potassium are present or induced.

In summary, these two studies further an understanding of how and why BBB opening leads to seizures and epileptogenesis. There now is overwhelming evidence that these mechanisms may have an important etiological role in acute or iatrogenic human seizures as well as in animal models. There are still several aspects to be elucidated, and consensus must be reached on how clinically relevant procedures (e.g., BBB disruption to treat brain tumors) and experimental approaches (e.g., bile salts, low concentrations of mannitol) can be reconciled. Perhaps, the most surprising findings of the study by van Vliet is the fact that epileptogenesis was induced by procedures that are clinically used to prevent seizures and neuronal damage. In any event, both studies demonstrate the urgent need for new strategies to improve BBB function or to prevent its breakdown during seizures.

by Damir Janigro, PhD

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Valproate Enhances Neuropeptide Y Expression: Modulating the Modulators

Chronic Valproic Acid Treatment Triggers Increased Neuropeptide Y Expression and Signaling in Rat Nucleus Reticularis Thalami. Brill J, Lee M, Zhao S, Fernald RD, Huguenard JR. *J Neurosci* 2006;26:6813–6822. Valproate (VPA) can suppress absence and other seizures, but its precise mechanisms of action are not completely understood. We investigated whether VPA influences the expression of neuropeptide Y (NPY), an endogenous anticonvulsant. Chronic VPA administration to young rats (300–600 mg · kg^{-1} · d^{-1} in divided doses over 4 d) resulted in a 30–50% increase in NPY mRNA and protein expression in the nucleus reticularis thalami (nRt) and hippocampus, but not in the neocortex, as shown by real-time PCR, radioimmunoassay, and immunohistochemistry. No increased expression was observed after a single acute dose of VPA. Chronic treatment with the pharmacologically inactive VPA analog octanoic acid did not elicit changes in NPY expression. No significant expression changes could be shown for the mRNAs of the Y_1 receptor or of the neuropeptides somatostatin, vasoactive intestinal polypeptide, and choleocystokinin. Fewer synchronous spontaneous epileptiform oscillations were recorded in thalamic slices from VPA-treated animals, and oscillation duration as well as the period of spontaneous and evoked oscillations were decreased. Application of the Y_1 receptor inhibitor *N*2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-d-arginine-amide (BIBP3226) enhanced thalamic oscillations, indicating that NPY is released during those oscillations and acts to downregulate oscillatory strength. Chronic VPA treatment significantly potentiated the effect of BIBP3226 on oscillation duration but not on oscillation period. These results demonstrate a novel mechanism for the antiepileptic actions of chronic VPA therapy.

COMMENTARY

The cellular basis of epileptic seizures often is referred to as an imbalance involving excess excitation and/or insufficient inhibition. While disturbed connectivity within circuits also is important, the concept of altered excitability is useful to understanding many features of epileptic seizures. Disruption of normal ion channel function and glutamate/GABA neurotransmission have well-documented roles in epilepsy, but other modulatory systems may help regulate the balance of excitability in neuronal circuits. Neuropeptide Y (NPY) is one particularly promising endogenous antiepileptic peptide. The recent paper by Brill et al. builds on previous work to show that the ability of valproate to alter thalamocortical excitability involves regulation of NPY expression within the thalamus.

NPY is a 36-amino–acid peptide that is widely distributed throughout the CNS. In normal brain, this protein is expressed exclusively in inhibitory neurons. Investigations using a combination of intracerebroventricular administration of NPY, NPY overexpression, and knockout animals have shown that this neuromodulator helps control a variety of functions, including feeding, stress response, and reproduction. Six types of NPY receptors (referred to as Y1–Y6) have been proposed on the basis of pharmacological experiments, but only Y 1, 2, 4, and 5 actually have been cloned and shown to form functional receptors in native rat and primate tissue. The majority of NPY receptors

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in the brain are of the Y1 or Y2 subtype, with lower levels of Y5 being expressed in some brain regions. As with other G-protein–coupled receptors, NPY receptors activate a variety of secondary messenger systems. However, as a rule, Y1 receptors in the thalamus and hippocampus act postsynaptically to activate G-protein coupled inwardly rectifying potassium channels, while Y2 receptors inhibit neurotransmitter release through suppression of presynaptic calcium channels.

In addition to its other functions, NPY helps regulate neuronal excitability and may be an important component in controlling the seizure threshold. As reviewed in a previous *Epilepsy Currents* commentary (1), work on multiple models of epilepsy has described the interrelationship between NPY and epileptic seizures. Intracranial administration of exogenous NPY suppresses seizures; similar results are obtained by using transgenic animals or recombinant viral vectors to overexpress NPY in the brain. Furthermore, inactivation of the *NPY* gene produces transgenic animals that, while largely normal, have lowered thresholds to both electrical and chemoconvulsant-induced seizures (2,3). Conversely, increased NPY expression is seen after acute seizures and chronic kindling in animal models of epilepsy (4) as well in tissue taken from epilepsy surgery patients with hippocampal sclerosis (5). Chronic epilepsy also is associated with more complex alterations of the NPY system, including upregulation of Y2 but decreased expression of Y1 receptors within the hippocampi (6). Finally, even the pattern of NPY expression is disturbed in the hippocampi of epileptic patients or animals. In normal subjects, NPY expression in the dentate gyrus is restricted primarily to hilar interneurons, with projections that include CA3 and the dentate molecular layer. Following status epilepticus, while inhibitory neurons of the dentate hilus are lost, there actually is increased NPY expression in the dentate molecular layer. As part of the pathological remodeling that occurs during temporal lobe epileptogenesis, dentate granule cells develop recurrent mossy fiber projections that express NPY de novo. This unique expression of NPY by a glutamatergic neuron may help restrain the hyperexcitable dentate granule cells through presynaptic inhibition of glutamate release (7). Indeed, consistent with the efficacy of NPY to suppress seizures, the recurrent excitation of dentate granule cells in slices from epileptic animals is reduced by application of Y2 agonists and enhanced by Y2 antagonists. While Y5 analogs also may have anticonvulsant activity, it is unclear how much of this effect actually is due to nonspecific activation of Y2 receptors (3). In contrast, similar studies have suggested that activation of Y1 receptors may lower the seizure threshold. Thus, NPY may either increase or decrease excitability, depending on the specific cell type and the NPY receptors involved.

In contrast to temporal lobe seizures, very little is known about the role on NPY in idiopathic generalized epilepsy. Spike-wave discharges, the electrical hallmark of absence seizures, are generated in the thalamocortical circuit, which includes the thalamic relay nuclei, neocortex, and the nucleus reticularis of the thalamus (nRT). Thalamic relay neurons send ascending excitatory projections to the cortex as well as to the nRT. Cortical neurons then send descending excitatory inputs back to the nRT. The nRT form a shell of exclusively GABAergic neurons around the rest of the thalamus. Each nRT neuron forms inhibitory synapses upon many thalamic relay cells. This recurrent circuit allows the simultaneous inhibition of many thalamic relay neurons, followed by a brief volley of rebound action potentials, thereby producing the synchronous, slow thalamocortical rhythms of sleep. Within the nRT itself, there are inhibitory interconnections that, when disrupted, can produce the hypersynchronous thalamocortical discharges of absence seizures. In addition to GABA, nearly all nRT neurons express NPY, and the nRT is the primary source of NPY input to the rest of the thalamus. The physiological role of NPY in the thalamus currently is unknown; however, recent work has sought to clarify this system. Investigators used a combination of NPY knockout animals with NPY analogs to show that burst firing in nRT neurons releases NPY, which subsequently activates Y1 receptors, causing a slow hyperpolarization via activation of G-protein inwardly rectifying potassium channels within the nRT neurons. Furthermore, application of NPY or the Y1 preferring peptide, [$Leu^{31}Pro^{34}$] NPY, partially suppressed the thalamic network oscillations induced by electrical stimulation in bicuculline-treated brain slices. The opposite effects were seen with application of the selective nonpeptide Y1 receptor antagonist, BIBP3226, suggesting that NPY is released endogenously during burst firing, thereby limiting the duration and/or synchrony of these bursts (8).

Idiopathic generalized epilepsies are unusual in that they are often insensitive to, or even exacerbated by, many of the more commonly used antiepileptic drugs. Valproate is one of the few medications that are efficacious for these patients. The mechanism of valproate action is still unclear, but it may involve changes in the activity of certain transcription factors that, thereby, regulate the expression of key neuronal proteins. Along these lines, preliminary work in cell culture had suggested that valproate might alter NPY expression. The paper by Brill et al. expands on earlier findings to explore the effect of subacute valproate treatment to alter NPY modulation of thalamocortical circuits. Following 4 days of valproate administration, there was an increase in NPY expression in the nRT and hippocampus but not in the neocortex. The physiological significance of these changes was explored in thalamocortical slice preparations taken from animals treated with valproate or the biologically inactive analog, sodium octanoate. While acute application of valproate to brain slices did not alter burst firing, slices from valproate-treated animals had reduced burst duration as well as reduced synchrony among cells during a burst.

Furthermore, BIBP3226 increased the duration of thalamic oscillations in control and valproate-treated animals, suggesting a tonic activation of Y1 receptors in burst firing nRT neurons. Moreover, the magnitude of this effect was significantly greater in slices from valproate-treated animals. Since there was no detectable change in Y1 receptor expression following valproate treatment, these effects likely are related to increased expression and/or release of NPY.

While intracerebroventricular injection of NPY suppresses spike-wave discharges in the genetic absence epilepsy rats from Strasbourg (GAERS) model of absence epilepsy (9), the role of NPY in idiopathic generalized epilepsy otherwise is almost completely unknown. It will be interesting to see whether genetic models of absence have disrupted NPY function, especially if they are responsive to clinically relevant antiepileptic medications. Conversely, while NPY knockout mice have seizure-like behavioral events (2), it is unclear whether disruption of NPY expression in specific brain regions can cause absence seizures. Furthermore, it is entirely possible that other, unknown components of the NPY system may help to regulate thalamocortical function. The work by Brill et al. focused on Y1 because it is the predominant NPY receptor type within the thalamus. However, Y2 and Y5 receptors also are present (10), and activation of thalamic Y2 receptors lowers the frequency of inhibitory postsynaptic currents through suppression of N/P-type calcium channels (11). Given that Y1-receptor activation may lower the threshold for some seizure types, it would be useful to know if Y2-receptor activation also limits thalamocortical excitability. Thus, while NPY may be important in normal thalamocortical functioning and epilepsy, much work remains to be done.

In addition to being a broad-spectrum antiepileptic medication, valproate causes a number of other therapeutic as well as adverse effects. In some patient populations, the weight gain associated with valproate treatment is particularly troublesome. Given the importance of NPY in the regulation of feeding, it is tempting to speculate that the orexic effects of valproate also involve enhanced NPY expression. Valproate treatment did not alter NPY expression in the hypothalamic paraventricular nucleus, a key player in the regulation of food intake. However, expression of NPY or its receptors were not determined in other feeding-related hypothalamic nuclei, and it remains unknown which, if any, of valproate's diverse effects actually are mediated by enhanced NPY expression. Furthermore, although NPY clearly has anticonvulsant effects on a variety of seizure models, the clinical utility of those finding is far from obvious. The lack of available NPY-specific drugs and the diverse actions of NPY on a variety of physiological functions make it unlikely that direct manipulation of NPY receptors will be useful in treating epilepsy in the near future. The findings of Brill et al. help expand our understanding of the role of neuropeptides to determine neuronal excitability, especially as it relates to the treatment of epilepsy. Perhaps more important, the ability of valproate to induce upregulation of a specific neuromodulatory peptide in specific brain regions provides an exciting alternative approach to the study and treatment of epilepsy patients.

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GLUTAMATE RECEPTORS: FINALLY FINGERED IN INHERITED EPILEPSY?

Epilepsy-Related Ligand/Receptor Complex LGI1 and ADAM22 Regulate Synaptic Transmission. Fukata Y, Adesnik H, Iwanaga T, Bredt DS, Nicoll RA, Fukata M. *Science* 2006;313(5794):1792–1795. Abnormally synchronized synaptic transmission in the brain causes epilepsy. Most inherited forms of epilepsy result from mutations in ion channels. However, one form of epilepsy, autosomal dominant partial epilepsy with auditory features (ADPEAF), is characterized by mutations in a secreted neuronal protein, LGI1. We show that ADAM22, a transmembrane protein that when mutated itself causes seizure, serves as a receptor for LGI1. LGI1 enhances AMPA receptor-mediated synaptic transmission in hippocampal slices. The mutated form of LGI1 fails to bind to ADAM22. ADAM22 is anchored to the postsynaptic density by cytoskeletal scaffolds containing stargazin. These studies in rat brain indicate possible avenues for understanding human epilepsy.

COMMENTARY

The role of ion channelopathy in inherited epilepsy continues to expand as new gene mutations underlying epilepsy syndromes are identified. Since the first study describing the linkage of autosomal dominant nocturnal frontal lobe epilepsy with the mutation of a gene encoding the nicotinic acetylcholine receptor (1), the number of epilepsy syndromes linked to single gene mutations has grown dramatically (2). If we consider the pure human epilepsy syndromes that lack other neurological or nonneurological phenotypes (e.g., excluding tuberous sclerosis and similar syndromes with associated cortical dysplasia and other pathologic features), it is remarkable that the identified genes have almost invariably encoded ion channels, whether voltage-gated or ligand-gated. In the voltage-gated channel category, dysfunctional sodium, potassium, calcium, and chloride channels have all been linked to inherited epilepsy, while $GABA_A$ and nicotinic acetylcholine receptors have been implicated among ligand-gated channels. (Curiously, the major excitatory glutamate-gated channels, AMPA and NMDA, have been absent from this list—but read on.) These findings reinforce the primary role of ion channel dysfunction in inherited epilepsy—a compelling pathogenic mechanism for what had been an idiopathic disease.

However, this almost perfect correspondence of inherited epilepsy and channelopathy has been marred by one notable outlier: the syndrome of autosomal dominant partial epilepsy with auditory features (ADPEAF). This syndrome is relatively rare but unmistakable when encountered in the clinic. Patients typically have secondarily generalized seizures that are preceded by unusual auditory auras (3). The aura may consist either of unformed sounds, such as a "machinery-like" whine that gradually increases in intensity before the convulsion, or of recognizable music or voices. Onset is typically in the teens or 20s, and the seizures are usually relatively easily controlled with medication. Spontaneous remission of seizures often occurs in later years. Inheritance is autosomal dominant with incomplete penetrance. The gene implicated in ADPEAF is the leucine-rich, glioma-inactivated 1 (*LGI1*) locus (4), which was initially described to be homozygously deleted in a subset of cerebral gliomas, suggesting that its product functions as a tumor suppressor.

While the link between the *LGI1* mutation and ADPEAF appears to break the one-to-one correspondence between ion channelopathy and epilepsy, several recent studies have delineated functions of the LGI1 protein that are unrelated to its putative tumor suppressor action. LGI1 is part of a family of genes, *LGI1-4*, also known as *epitempin*. Analysis of their protein structures suggests that they lack the transmembrane domains typical of ion channels. Rather, the structures predict a secreted protein, and in vitro evidence shows that LGI1 and other family members are secreted when exogenously expressed (5). Typical *LGI1* mutations seen in ADPEAF would be predicted to cause truncation of the expressed protein and do in fact reduce their secretion or their extracellular stability. Thus, the mutations seen in ADPEAF would be expected to produce a loss-of-function of the LGI1 protein.

But what is that function? The current paper by Fukata et al. (6) discovers a role for LGI1 that completes the link between ADPEAF and channelopathy. The investigators started by screening for proteins associated with the postsynaptic density protein-95 (PSD-95). As its name implies, PSD-95 is a major constituent of the neuronal membrane area juxtaposed to the synaptic cleft on the postsynaptic side. It functions as a backbone for a variety of synaptic proteins (including glutamate-gated ion channels), their regulatory subunits, and downstream signaling molecules. When the authors isolated PSD-95 from neuronal membranes, they principally found three tightly associated proteins: LGI1, stargazin, and ADAM22. Stargazin is a protein that mediates insertion of AMPA receptors into the postsynaptic membrane by anchoring them to PSD-95 (7); interestingly, it is mutated in the stargazer mouse strain with absence epilepsy and ataxia. ADAM22 is a member of a large

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family of transmembrane proteins, and it too is tied to PSD-95 on its intracellular end but also traverses the membrane to protrude into the extracellular space, possibly functioning as a cell adhesion molecule. Fukata and colleagues demonstrated that LGI1 binds to the extracellular portion of ADAM22; this binding in turn appears to increase the number of AMPA receptors inserted into the postsynaptic membrane, augmenting excitatory neurotransmission. Loss of LGI1 function, as seen in ADPEAF, would thus be expected to reduce glutamatergic neurotransmission via AMPA receptors.

The results of Fukata et al. provide a molecular mechanism for the genetic defect seen in ADPEAF. This exciting finding potentially adds glutamate receptor dysfunction to the list of human epileptic channelopathies and strengthens the association between inherited epilepsy and ion channelopathy. But as may be expected for a result this novel, more questions are generated than can be immediately answered. Loss of LGI1 function as would occur in ADPEAF would be predicted to reduce synaptic AMPA receptors, much as mutant stargazin does in epileptic mice, but this hypothesis remains to be proven, and doing so may depend on the generation of mice with *LGI1* deletion. Why the defects in AMPA receptor trafficking seen (or predicted) in *stargazin* and *LGI1* mutations would produce epilepsy is not immediately clear—much less why they would cause such disparate forms of epilepsy in mice (generalized seizures) versus humans (focal onset seizures). And, considering that the distributions of LGI1 and its partner-in-crime ADAM22 appear widespread throughout the cortex (among other structures), why does the *LGI1* mutation in ADPEAF cause seizures with such apparently focal onset in lateral temporal neocortex? Finally, the demonstration of a biological mechanism is of course not the proof that it is sufficient to cause the disease phenotype. An additional interaction has been proposed for LGI1 in the modulation of Kv1.1 channels (8). As the loss of these voltage-gated channels has been associated with epilepsy in animal models, this finding too might be a plausible mechanism in human epilepsy. Confirmation of the biological relevance of these mechanisms in epilepsy almost certainly will require further work using animal models.

The present work, nonetheless, is important for delving into the molecular roots of neuronal excitability to discover the causes of human epilepsy. That this path of investigation again leads to ion channelopathy suggests that ion channel dysfunction is the primary basis of inherited human epilepsy syndromes. One might wonder whether such channelopathy mechanisms will be found to underlie the various acquired forms of epilepsy as well.

by Nicholas P. Poolos, MD, PhD

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THE BEST MODEL FOR A CAT IS THE SAME CAT. . .OR IS IT?

Effect of Antiepileptic Drugs on Spontaneous Seizures in Epileptic Rats. Nissinen J, Pitkänen A. *Epilepsy Res* 2007;73: 181–191. The present study investigated whether spontaneously seizing animals are a valid model for evaluating antiepileptic compounds in the treatment of human epilepsy. We examined whether clinically effective antiepileptic drugs (AEDs), including carbamazepine (CBZ), valproic acid (VPA), ethosuximide (ESM), lamotrigine (LTG), or vigabatrin (VGB) suppress spontaneous seizures in a rat model of human temporal lobe epilepsy, in which epilepsy is triggered by status epilepticus induced by electrical stimulation of the amygdala. Eight adult male rats with newly diagnosed epilepsy and focal onset seizures were included in the study. Baseline seizure frequency was determined by continuous video-electroencephalography (EEG) monitoring during a 7 days baseline period. This was followed by a 2–3 days titration period, a 5–7 days treatment period, and a 2–3 days wash-out period. During the 5–7 days treatment period, animals were treated successively with CBZ (120mg/kg/day), VPA (600mg/kg/day), ESM (400mg/kg/day), LTG (20mg/kg/day), and VGB (250mg/kg/day). VPA, LTG, and VGB were the most efficient of the compounds investigated, decreasing the mean seizure frequency by 83, 84, and 60%, respectively. In the VPA group, the percentage of rats with a greater than 50% decrease in seizure frequency was 100%, in the LTG group 88%, in the VGB group 83%, in the CBZ group 29%, and in the ESM group 38%. During the 7 day treatment period, 20% of the VPA-treated animals and 14% of the CBZ-treated animals became seizure-free. These findings indicate that rats with focal onset spontaneous seizures respond to the same AEDs as patients with focal onset seizures. Like in humans, the response to AEDs can vary substantially between animals. These observations support the idea that spontaneously seizing animals are a useful tool for testing novel compounds for the treatment of human epilepsy.

COMMENTARY

In recent years, intense discussion has evolved around the question of which experimental models are better suited for studying human epilepsy. For example, recommendations for the development of epilepsy models have been outlined at two NIH workshops (1,2); analyzed in both opening and closing chapters of the book *Models of Seizures and Epilepsy* (3,4); and most recently, were a subject of heated debate at an Investigator's Workshop session of the 1st North American Regional Epilepsy Congress in San Diego, California (5). The major reason for the debate is to close the gap between bench and bedside through development of standardized test systems for clinically predictable, high-throughput screening of prospective antiepileptic drugs (AEDs). Furthermore, the discussion reflects different and often conflicting viewpoints on "what good are animal models?" (4). These differences generally indicate a preference toward one of two approaches.

One approach is referred to as analogical modeling; it is based on the maxim, "the best material model for a cat is another, or preferably the same cat" (6). This approach contends that the more an animal's condition resembles human epilepsy, the closer the former reflects the latter. From this perspective, models such as pentylenetetrazole seizures, maximal electroshock, and kindling have very limited clinical relevance, as they clearly fail the analogy test. At the same time, models that are characterized by spontaneous seizures, such as post–status epilepticus or posttraumatic epilepsy in rats, are considered to be more compelling. A second approach, conceptual modeling, is best embodied by a René Magritte's painting "The Treachery of Images," in which a picture of a pipe is accompanied by the subtitle "this is not a pipe" (meaning: this is only an image, not a pipe). Conceptual modeling asserts that a model cannot merely bear a resemblance to a subject but rather has to reproduce sufficiently the subject or the process of interest.

The difference between the two approaches is obvious. While analogical models strive to encompass all factors of the human condition, conceptual models are explicitly incomplete regarding some details (i.e., idealized). A key rationale underlying the conceptual model is to establish logical relationships among variables rather than simply to account for as many variables as possible. Idealization is a key feature of the conceptual model, allowing for simplification of the phenomenon to such an extent that it can be studied effectively. From the practical standpoint, idealization also permits more efficiency, which in the case of AED development translates into high throughput of a large number of prospective AEDs within a reasonable time frame and at an affordable cost. Presently, basic epilepsy research offers a large variety of animal models; consequently, model development has focused on validation of existing models to select which ones are most relevant for either basic (studies of mechanisms) or translational (development of diagnostic and treatment tools) research.

The manuscript by Nissinen and Pitkänen is an example of validation of an animal model for translational research. The authors attempted to answer the question of whether spontaneous, recurrent seizures that develop in rats after status epilepticus may be used as a tool for identifying prospective AEDs.

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They tried to combine advantages of analogical and conceptual approaches by adopting a multifaceted phenomenon (i.e., post–status epilepticus chronic epilepsy) and by simplifying this phenomenon through reducing the number of the parameters presumed to be indicative of AED efficacy in humans. The study design was based on the assumption that if the AED profile of the post–status epilepticus model in rats is similar to that in human temporal lobe epilepsy, it might be a good model to screen human AED efficacy. The authors chose five AEDs with known efficiency in human temporal lobe epilepsy and examined how they worked in rat epilepsy.

Post–status epilepticus epilepsy in rats includes a wide assortment of variables. Spontaneous seizures per se vary in terms of frequency, duration, and severity, both among the animals and within the same animal. Interictal changes include spikes, high frequency oscillations, and behavioral deficits, such as cognitive, memory, and mood impairments. Clearly, when assessing the effectiveness of AEDs, all these features are difficult, if not impossible, to take into the account. To simplify the analysis, Nissinen and Pitkänen selected just two symptoms of epilepsy: seizure frequency and seizure duration. They found that by and large the variability of spontaneous seizures as well as their responsiveness to AEDs was similar to human temporal lobe epilepsy. Hence, the investigators assumed that the drugs that perform best in this model also are the best AEDs in human epilepsy. Did the study succeed? Do the results suggest that post–status epilepticus epilepsy in animals indeed represents the best system for AED screening for temporal lobe epilepsy?

The authors state that the variability of analyzed parameters (both baseline and in response to AED treatment) is an advantage, since rat epilepsy can be used "to mimic clinical study designs of preclinical trials." Thus, from the analogical modeling standpoint, the validation process was a success. However, as discussed, the very same features that are advantageous in analogical modeling represent substantial flaws for conceptual models. The latter would prefer uniformity to variability in both seizure phenotype and AED effects. The authors admitted that additional tuning of the model might be necessary, for example, through selective examination of animals with "severe" versus "mild" epilepsy. Further scrutiny of the model also might be useful, including examination of the effects of prospective of AEDs on seizure prevention versus seizure spread; modification of interictal epileptic phenomena, such as spikes; and improvement in nonconvulsive comorbidities, such as cognition, memory, and mood disorders. Development of alternative treatment protocols and optimization of evaluation criteria also should be explored (7).

Then again, is it worth pursuing other models for the development and validation of AED screening? It has been correctly emphasized that depending on the purpose (e.g., drug discovery versus mechanistic studies), models for the same condition may and probably should be different (2). Thus, translational epilepsy research does not have to limit itself to models that have similar epidemiological and clinical characteristics to those under conditions of human epilepsy.

An appeal of post–status epilepticus epilepsy is that seizures develop in a seemingly spontaneous and erratic fashion, thus resembling the human condition. The vast majority of other models require seizure induction by certain external stimuli. However, the differences between post–status epilepticus epilepsy and other types of models are not necessarily as significant as they might seem. For example, under the conditions of the kindling model, the ratio of seizure response (overt secondary generalized seizures) to the strength of the applied stimulus (very low current, which is subconvulsant in naïve animals) is very high. At the same time, the assertion that seizures in post–status epilepticus models seizures are spontaneous is not necessarily correct. Indeed, seizures depend on circadian rhythms as well as minute fluctuations of concentrations of K^+, Ca^{2+}, hormones, and other factors. In effect, they likely are induced by a variety of both accounted and unaccounted for endogenous stimuli. Yet, kindling has an obvious advantage over spontaneous seizure models, as it offers full control over seizure induction—seizures only develop when needed for the given study design. Thus, no long-term monitoring with expensive equipment is required, and both the variable and erratic nature of seizure occurrence is easily avoided. More importantly, AED profiles of kindling and post–status epilepticus epilepsy are strikingly similar (8). Therefore, while kindling might not be a very good model for mechanistic and histopathological studies, it represents a viable alternative to spontaneous seizure epilepsy for the purpose of AED testing.

In summary, the study by Nissinen and Pitkänen emphasizes that choosing and validating an epilepsy model is a not a trivial task. Selection of appropriate parameters for analysis and criteria for the efficacy of AEDs is far from complete. Furthermore, the pursuit of more "user-friendly," yet clinically relevant, models is not to be forgotten.

by Andrey Mazarati, MD, PhD

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Is Too Much Inhibition to Blame in Autosomal Dominant Nocturnal Frontal Lobe Epilepsy?

Seizures and Enhanced Cortical GABAergic Inhibition in Two Mouse Models of Human Autosomal Dominant Nocturnal Frontal Lobe Epilepsy. Klaassen A, Glykys J, Maguire J, Labarca C, Mody I, Boulter J. *Proc Natl Acad Sci U S A* 2006;103(50):19152–19157. Selected mutations in the human α4 or β2 neuronal nicotinic acetylcholine receptor subunit genes cosegregate with a partial epilepsy syndrome known as autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). To examine possible mechanisms underlying this inherited epilepsy, we engineered two ADNFLE mutations (*Chrna4*^{S252F} and *Chrna4*$^{+L264}$) in mice. Heterozygous ADNFLE mutant mice show persistent, abnormal cortical electroencephalograms with prominent delta and theta frequencies, exhibit frequent spontaneous seizures, and show an increased sensitivity to the proconvulsant action of nicotine. Relative to WT, electrophysiological recordings from ADNFLE mouse layer II/III cortical pyramidal cells reveal a >20-fold increase in nicotine-evoked inhibitory postsynaptic currents with no effect on excitatory postsynaptic currents. i.p. injection of a subthreshold dose of picrotoxin, a use-dependent γ-aminobutyric acid receptor antagonist, reduces cortical electroencephalogram delta power and transiently inhibits spontaneous seizure activity in ADNFLE mutant mice. Our studies suggest that the mechanism underlying ADNFLE seizures may involve inhibitory synchronization of cortical networks via activation of mutant α4-containing nicotinic acetylcholine receptors located on the presynaptic terminals and somatodendritic compartments of cortical GABAergic interneurons.

COMMENTARY

Cholinergic projections, originating primarily in the basal forebrain, influence neuronal excitability throughout the cerebral cortex and hippocampus. Although extensive, the projections are sparsely distributed, making detailed physiological studies of the effects of cholinergic inputs difficult, and therefore the precise functions of the cholinergic system are not well understood. In general, activity of cholinergic neurons correlates with cortical activation during wakefulness and REM sleep (1). Acetylcholine acts at both ionotropic nicotinic acetylcholine receptors (nAChRs) and metabotropic muscarinic acetylcholine receptors (mAChRs). mAChRs influence a variety of important brain processes, such as attention, memory, and the sleep/wake cycle. Pilocarpine, a muscarinic agonist, causes seizures in high doses and is used to generate status epilepticus in a widely studied animal model. An important role for nAChRs in seizures and epilepsy was confirmed by the association of mutations in certain nAChR genes in a hereditary form of epilepsy, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE).

Twelve different nAChR subunits (α2–10 and β2–4) have been identified that may combine to form pentameric ligand-gated, cation-selective channels. Based on subunit homologies and proposed structural similarities, nAChRs belong to a family that includes the $GABA_A$ receptors. Of the many possible subunit combinations, only $\alpha4\beta2$- and homomeric α7-containing receptors appear to be expressed at high levels in the brain. These two receptor configurations are characterized by high- and low-affinity binding of agonist, respectively. Like the $GABA_A$ receptor, after opening, the nAChR rapidly enters a closed, desensitized state. Unlike the $GABA_A$ receptor, which contains a chloride channel, nAChR activation results in a brief depolarizing, excitatory potential. The nAChRs also have variable permeability to Ca^{2+} ions, enabling them to influence intracellular signaling pathways in addition to their depolarizing effects.

Because of the relative paucity of nAChR-containing postsynaptic sites identified in anatomical studies, it has been proposed that the majority of signaling mediated by nAChRs occurs via "volume transmission," that is, via activation of receptors at

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nonsynaptic sites (2). Most acetylcholine signaling in the brain appears to be mediated through its action at presynaptic terminals, where it depolarizes and/or increases calcium influx to enhance neurotransmitter release. Although acetylcholine has been shown to increase release of many neurotransmitters, the evidence for nAChR-mediated presynaptic effects is strongest at GABAergic neurons. There is evidence of localization of nAChRs to other subcellular sites, including dendrites and somata, and many other roles in modulating neuronal excitability have been suggested (2).

In 1995, a missense mutation in the $\alpha 4$ subunit gene (*CHRNA4*) was found to underlie ADNFLE, and subsequently five additional mutations both in the $\alpha 4$ and $\beta 2$ subunit (*CHRNB2*) genes were associated with the same disease (3). ADNFLE is characterized by hyperkinetic seizures that occur mostly during non-REM sleep. All of the identified disease-causing mutations are located near the proposed pore of the ion channel, and electrophysiological studies of the mutated receptors have revealed a variety of altered properties, including decreased Ca^{2+} permeability in some mutant receptors and increased desensitization in others. One common finding among these mutations is that sensitivity to acetylcholine is increased (4). Extensive studies of the mutant channels have failed to offer a single mechanistic explanation for the clinical manifestations of the mutations. One of the more interesting questions that arose from the investigations is why mutations in a receptor that is widely expressed throughout the brain cause seizures with a focal onset in the frontal lobes. The implication of the results of earlier studies was that altered nAChR function affects local neuronal network behavior in a complex manner that cannot be explained by channel properties alone (3).

The recent work by Klaassen et al. is a major advance in our understanding of the pathophysiology of ADNFLE. These researchers engineered two mouse lines with mutations in the $\alpha 4$ subunit, *Chrna4*^{S252F} (an amino acid exchange) and *Chrna4*$^{+L264}$ (an insertional mutation), which correspond to those in human families. The heterozygous mice were studied in detail because this genotype replicates the human condition in ADNFLE. Both mutant strains of mice had abnormal EEGs, characterized by increased slow activity and repetitive spontaneous seizures associated with sudden onset of rhythmic high-voltage, low-frequency, and asymmetric spike-and-wave discharges. They also demonstrated an increased susceptibility to nicotine-induced seizures.

In an effort to determine the cellular physiological changes underlying the epileptic phenotype, whole cell recordings were performed in cortical pyramidal neurons in brain slices from the mutant mice. No changes in frequency or amplitude of spontaneous EPSCs or IPSCs were observed under baseline conditions when compared with wild-type controls. However, application of nicotine to the brain slices from ADNFLE mice, but not wild type, revealed a dramatic and selective effect on IPSCs. The amplitude and frequency of spontaneous IPSCs were increased by nicotine in ADNFLE mice, and the net effect was given a quantitative value by calculating the mean inhibitory current as a function of time. As expected for a nAChR-mediated effect, nicotine produced an increase in the mean inhibitory current, which decayed during continued application of the drug, presumably corresponding to activation followed by desensitization of the receptors. Nicotine created approximately a 20-fold increase in the mean inhibitory current in both mutant strains, compared with a 2.5-fold increase in neurons from wild-type mice. Using selective agonists and antagonists, Klaassen et al. argue that the enhanced nicotine response was mediated by $\alpha 4\beta 2$ receptors. To determine the mechanism by which nicotine increased GABAergic output, it was applied to slices after blocking both voltage-gated sodium channels (with TTX) and calcium channels (with cadmium). Under these conditions, there was no difference in the occurrence of spontaneous miniature IPSCs (mIPSCs) between wild-type and ADNFLE mice, neither was there any change in the occurrence of mIPSCs in the presence of nicotine in wild-type mice. However, nicotine increased the frequency and amplitude of mIPSCs in ADNFLE mice. To explain these combined findings, the authors suggest that mutant nAChRs mediate a presynaptic elevation of Ca^{2+} in the terminals of inhibitory neurons, facilitating the release of GABA-containing synaptic vesicles from their release sites.

The findings of Klaassen and colleagues, therefore, suggest that an exaggerated effect of acetylcholine on presynaptic nAChRs enhances the release of GABA in ADNFLE mutants. To confirm the seemingly paradoxical finding that increased inhibitory output in the cortex could underlie the generation of seizures in ADNFLE mice, a $GABA_A$ receptor antagonist, picrotoxin, was administered in doses low enough to have no effect on wild-type mice. ADNFLE mice, in contrast, showed a normalization of their EEGs and a cessation of spontaneous seizures.

The authors propose a model in which GABAergic interneurons innervate a network of cortical pyramidal neurons. Acetylcholine, acting through presynaptic nAChRs, transiently enhances the release of GABA and causes a strong inhibition that, when relieved, results in a synchronization of the pyramidal network output. The effect of acetylcholine is greatly enhanced in the ADNFLE mutants, resulting in hypersynchronization and seizures. The adjunct experiments showing that picrotoxin, which normally has convulsant properties as a result of its effect on $GABA_A$ receptors, actually normalized the EEG and stopped seizures is strong evidence that this model is correct. Currently, however, there is no direct evidence that

acetylcholine released from cholinergic projections can synchronize populations of pyramidal neurons. Moreover, this model will have to be reconciled with the models of cholinergic activity corresponding to arousal, because nocturnal frontal lobe seizures are most common in stage 2 sleep. The findings in this study provide new insights into a type of partial epilepsy that, although caused by a mutation in a widely distributed receptor, may arise from a complex interaction involving cholinergic modulation of specific interneuron populations and excitatory neuronal networks. If true, the findings also may have implications for other forms of epilepsy and their treatment. The idea that some GABAergic neurons have more of a proepileptic than an antiepileptic function is not new. However, the idea that distinct populations of interneurons may respond differently to drugs, such as nicotine, to modulate cortical excitability raises the possibility that new antiepileptic drug strategies could exploit these mechanisms.

by Gregory C. Mathews, MD, PhD

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The Epileptic Hippocampus Revisited: Back to the Future

Massive and Specific Dysregulation of Direct Cortical Input to the Hippocampus in Temporal Lobe Epilepsy. Ang CW, Carlson GC, Coulter DA. *J Neurosci* 2006;26(46):11850–11856. Epilepsy affects 1–2% of the population, with temporal lobe epilepsy (TLE) the most common variant in adults. Clinical and experimental studies have demonstrated hippocampal involvement in the seizures underlying TLE. However, identification of specific functional deficits in hippocampal circuits associated with possible roles in seizure generation remains controversial. Significant attention has focused on anatomic and cellular alterations in the dentate gyrus. The dentate gyrus is a primary gateway regulating cortical input to the hippocampus and, thus, a possible contributor to the aberrant cortical-hippocampal interactions underlying the seizures of TLE. Alternate cortical pathways innervating the hippocampus might also contribute to seizure initiation. Despite this potential importance in TLE, these pathways have received little study. Using simultaneous voltage-sensitive dye imaging and patch-clamp recordings in slices from animals with epilepsy, we assessed the relative degree of synaptic excitation activated by multiple cortical inputs to the hippocampus. Surprisingly, dentate gyrus-mediated regulation of the relay of cortical input to the hippocampus is unchanged in epileptic animals, and input via the Schaffer collaterals is actually decreased despite reduction in Schaffer-evoked inhibition. In contrast, a normally weak direct cortical input to area CA1 of hippocampus, the temporoammonic pathway, exhibits a TLE-associated transformation from a spatially restricted, highly regulated pathway to an excitatory projection with >10-fold increased effectiveness. This dysregulated temporoammonic pathway is critically positioned to mediate generation and/or propagation of seizure activity in the hippocampus.

COMMENTARY

The hippocampus is considered by many to be the generator of temporal lobe epilepsy (TLE). This view largely is due to the frequent observation of the histopathology of sclerosis in the Sommer's sector and in the endfolium of the hippocampus of TLE patients. In addition, surgical removal of the sclerotic hippocampus often improves this epileptic condition (1). However, several aspects of TLE pathophysiology remain elusive, and even the role of hippocampal sclerosis is unsettled. Almost 13 years ago, Pierre Gloor expressed this mindful conviction in a letter addressed to Dan McIntyre, stating: ". . .even though we know that most temporal lobe seizures in humans originate from the mesial structures, we are far from understanding which structures are essential or play what role, which is or are the sites of seizure onset and which are the routes of propagation of the seizure discharge. There has been, in my opinion, a simplistic view that the hippocampus is possibly the sole center of action. Hippocampal sclerosis is certainly the most outstanding neuropathological finding in resected temporal lobes of temporal lobe epileptics. And since patients with proven hippocampal sclerosis do best after surgery, the conclusion was that is the sclerotic hippocampus that is the site of origin of the seizures. This may be so, but remains unproven

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and there are difficulties with this explanation (2)." Then, he continued: "The experimental neurophysiologists who work on normal hippocampi consistently identify CA3 as the site of origin of discharge in a variety of models of experimental hippocampal epilepsy. . .. It is hard to see how in an abnormal, sclerotic hippocampus this could be the mechanism of seizure genesis and propagation with hardly any neurons left in either CA3 or CA1 (2)."

To date, investigations on the pathophysiogenesis of TLE mainly have focused on the role of the dentate gyrus in gating the arrival of the epileptic discharge to the hippocampus. The dentate gyrus is the obligatory route by which impulses reach the hippocampus and are elaborated to regain access to the limbic cortices through the trisynaptic pathway (i.e., the loop composed of the entorhinal cortex→dentate gyrus→CA3→CA1-subiculum and back again to entorhinal cortex). Indeed, in the epileptic hippocampus, the dentate gyrus undergoes changes consisting of the loss of dentate hilus interneurons, appearance of newly formed ectopic granule cells, and sprouting of mossy fibers, thus, suggesting a high remodeling of dentate-hippocampal circuits in strict correlation to epileptogenesis (3). However, the recent paper published by Ang and colleagues appears to limit the role of the dentate gyrus in TLE, as it shows that this hippocampal structure has comparable responses in both epileptic and control rats.

These authors addressed the role of the dentate gyrus in epileptogenesis by comparing control and pilocarpine-treated epileptic rats; the latter present with electrographical and neuropathological abnormalities that are similar to those of TLE patients. The fact that activation of the dentate gyrus occurs in epileptic animals to a degree similar to what is seen in controls suggests that the gate-keeping function of dentate gyrus is maintained in epileptic rats. In addition, Ang et al. found low degrees of activation in the CA3 of both animal groups. Since the CA3 pyramidal layer is activated by stimulating the Schaffer collaterals antidromically, the authors proposed that lack of CA3 hyperactivity (at least in pilocarpine-treated epileptic rats) cannot be explained by CA3 damage. Interestingly, a similar finding recently was reported in the same TLE model by imaging the intrinsic optical signals evoked by direct CA3 activation (4).

According to Gloor's comment (2), CA1 damage also could impair hippocampal output activity, because even when CA3 is intact, to be effective the epileptic discharge must be transmitted through CA1 to reach the other hippocampal regions. Far from being hypoactive, Ang and coworkers found a dramatic increase of the synaptic excitatory responses of CA1 networks. However, such a finding was unrelated to CA3 activity as it depended upon inputs arriving to CA1 from a network alternative to the classic trisynaptic pathway, that is, the temporoammonic pathway (5). This pathway originates in layer III of the entorhinal cortex, which is known to initiate limbic seizures both in TLE patients (6) and in animal models of epileptiform synchronization (5). Moreover, as properly discussed by these investigators, since temporoammonic inputs travel directly to the CA1 area, the transformation of the responses of CA1 pyramids from predominantly inhibitory to powerfully excitatory can supplement an efficacious reverberating loop that is well suited for sustaining seizure activity.

Some findings reported in this paper, however, are not fully addressed by Ang and colleagues. The first relates to the reduction in downstream transmission from CA3 to CA1, tested here by activating the Schaffer's collaterals. This evidence is in line with the finding of an impaired ability of CA3 and CA1 networks to generate pharmacologically induced interictal activity after status epilepticus (5) as well as with recent in vitro and ex vivo results indicating that the pilocarpine-treated CA3 area is less excitable than in controls (4). This characteristic may be relevant to TLE, as hypofunctional CA3/CA1 outputs may be unable to control entorhinal cortex excitability while contributing to the transformation of the responses of CA1 neurons to temporoammonic activation (5). Possible explanations for the finding include: (a) changes in the intrinsic properties of CA3 pyramidal neurons, and if true, then it would be important to know why such modifications are specific of this hippocampal area; and (b) the presence of an inhibitory tone contributed by dentate gyrus afferents, as suggested by experiments conducted in other animal models of TLE (7). These phenomena remain to be explored in the pilocarpine model.

The second finding by Ang and colleagues deserving discussion is that stimulation of the perforant pathway induces similar dentate excitatory responses in control and epileptic slices. The investigators concluded that the dentate area retains its gatekeeper role in this animal model of TLE; however, such a conclusion is unexpected because in both epileptic animals and humans, structural and functional changes occur in this area (8). Why these changes (i.e., sprouting in the inner molecular layer and interneuron loss in the hilus) are unable to alter the response to inputs arriving from the perforant path awaits an explanation. Alternatively, from these studies, it could be proposed that remodeling of dentate gyrus networks is oriented to the maintenance of the gate-keeping function.

As acknowledged by Ang and colleagues, their findings await verification in in vivo animal models of TLE. Nonetheless, they yield meaningful support to the hypothesis that changes in excitability restricted to defined areas of the limbic system and even to specific inputs contribute to epileptogenesis. Within this context, it is reasonable to anticipate that future studies on changes in excitability that characterize different epileptic limbic areas as well as the interactions among them can provide new

insight into therapeutic approaches that may be implemented in TLE.

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