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TREKKING THROUGH THE TELENCEPHALON: HEPATOCYTE GROWTH FACTOR-MEDIATED GUIDANCE FOR PARVALBUMIN-EXPRESSING INTERNEURONS

Hepatocyte Growth Factor (HGF) Modulates GABAergic Inhibition and Seizure Susceptibility. Bae MH, Bissonette GB, Mars WM, Michalopoulos GK, Achim CL, Depireux DA, Powell EM. *Exp Neurol* 2010;221(1):129–135. Disrupted ontogeny of forebrain inhibitory interneurons leads to neurological disorders, including epilepsy. Adult mice lacking the urokinase plasminogen activator receptor (*Plaur*) have decreased numbers of neocortical GABAergic interneurons and spontaneous seizures, attributed to a reduction of hepatocyte growth factor/scatter factor (HGF/SF). We report that by increasing endogenous HGF/SF concentration in the postnatal *Plaur* null mouse brain maintains the interneuron populations in the adult, reverses the seizure behavior and stabilizes the spontaneous electroencephalogram activity. The perinatal intervention provides a pathway to reverse potential birth defects and ameliorate seizures in the adult.

COMMENTARY

The connection between defects in GABAergic networks and epilepsy is well recognized. Recent reports have addressed the issue of whether altered development of excitatory and inhibitory networks could produce disturbances in cortical function in affective disorders of childhood and adolescence, including anxiety, depression, and schizophrenia (1,2). Mounting evidence indicates that there are genetic mutations that interfere with interneuron migration during brain development. Most forebrain GABAergic interneurons arise from proliferative regions in the basal telencephalon, called the ganglionic eminences. After exiting the cell cycle, newborn interneurons trek through the wide expanse of the basal forebrain to reach their final destinations in the cerebral cortex, hippocampus, amygdala, and striatum. Knowledge of how they arrive at these locations is far from complete. Sorting out the migratory mechanisms will be quite an undertaking, as GABAergic interneurons are functionally diverse, with more than 20 subclasses that show distinctive connectivity, neurochemistry, and functional properties. A new study by Bae et al., reviewed here, takes an important step in this direction. These authors used genetically altered mice to show that the absence of a key regulatory molecule, hepatocyte growth factor/scatter factor (HGF/SF), disrupts interneuron migration (3). This member of the plasminogen-related growth factor family has potent effects on cell motility.

Insights into the impact of disrupting the delicate ratio of excitatory and inhibitory neurons came unexpectedly from mice with targeted mutations of the gene *Plaur*, encoding the urokinase plasminogen activator receptor (uPAR) (4,5). When uPAR binds its ligand, uPA, the resultant proteolytic cascade activates HGF/SF. Loss of uPAR expression in *Plaur*-null mice diminishes HGF/SF levels. These mice show regional deficits in cortical GABAergic interneurons, spontaneous seizures, lower thresholds for chemically induced convulsions, and anxiety-related behaviors. *Plaur*-null mice survive into adulthood, making this mouse model ideal for exploring the roles of HGF/SF and its receptor Met (Met proto-oncogene or hepatocyte growth factor receptor) in interneuron migration, as *Hgf*- or *Met*-null mice die during embryonic life (6). HGF was initially identified in the liver, where it promotes hepatocyte proliferation and regeneration by binding to MET, a tyrosine kinase receptor (7,8). Scatter factor is a molecule produced by fibroblasts that initiates cell dispersion in epithelial-derived cells.

One remarkable finding in the *Plaur*-null mice is that the attendant decrease in HGF/SF levels chiefly impacts interneurons expressing the calcium-buffering protein parvalbumin (PV), while leaving unscathed the calretinin or somatostatin-expressing interneurons. The mice show a 50% drop in PV-immunoreactive (PV^+) interneurons in the parietal and cingulate cortices, while PV^+ interneurons in other cortical areas are unaltered. The cortical and hippocampal PV^+ interneurons comprise approximately 20% of the entire forebrain's GABAergic cells and are well described. Within the cerebrum and hippocampus, PV^+ interneurons exhibit fast-spiking action potentials, form perisomatic synaptic baskets onto neighboring neurons, and are responsible for generating gamma oscillations in the hippocampus. Experimentally disrupting these interneurons impairs hippocampal-related tasks, such as spatial working memory and novel object recognition (9).

A second key observation by Bae et al. in *Plaur* mice is that the PV^+ interneuron deficiency is limited to cingulate and parietal cortex (10). Several possible explanations are

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apparent. These areas might require higher levels of HGF/SF activity to guide migrating PV^+ interneurons, or other types of interneurons might use different migratory cues. To explore the underlying mechanisms, Bae et al. tested whether HGF/SF overexpression could rescue the mutant phenotype of *Plaur*-null mice. A transgenic mouse line expressing human HGF/SF under the control of the mouse glial fibrillary acidic protein (GFAP) promoter (*Gfap*-HGF mice) was crossed to the *Plaur*-null mice (HGF/*Plaur*-null mice), to drive expression of HGF/SF postnatally. Their results demonstrate a striking rescue of GABAergic interneurons in cingulate and parietal cortices in HGF/*Plaur*-null mice.

Because PV is regulated in an activity-dependent manner, it was necessary to demonstrate that *Plaur* mice show a true deficit in PV^+ interneurons, rather than reduced PV expression. The authors used an alternative method for identifying PV^+ interneurons based on the presence of "perineuronal nets" that are fenestrated sheaths surrounding PV^+ basket cell somas and initial axon segments. The nets contain extracellular matrix molecules with a special carbohydrate group, called *N*-acetylgalactosamine, which may stabilize synapses during postnatal critical periods. Staining brain sections with plant lectins conjugated to a fluorescent molecule or peroxidase allows visualization of these nets with light microscopy. Lectin staining of *Plaur*-null mouse brains verified that perineuronal nets are also absent. These experiments show that the apparent deficit in PV^+ interneurons stems from an actual reduction in the interneurons, rather than a failure of these neurons to express detectable amounts of PV. Bae et al. then established that the perineuronal nets surrounding basket cells are normally formed in the HGF/*Plaur*-null mice, confirming that genetically increasing levels of HGF/SF not only establishes molecular guidance cues for PV^+ interneurons but also allows them to develop extracellular matrix constituents that may be involved in synaptic stabilization.

In rodent epilepsy models, seizure susceptibility is often evaluated with a single threshold injection of pentylenetetrazole. Functional deficits in inhibitory networks can be unmasked with a subthreshold dose of pentylenetetrazole. While the majority of *Plaur* mice develop motor seizures after doses of pentylenetetrazole that are normally at subthreshold, less than 20% of the HGF or the HGF/*Plaur* mice showed seizures. Interestingly, *Plaur* mice exhibit abnormal baseline cortical activity, with episodes of high-amplitude spiking. While the significance of the abnormal interictal spikes is unknown, this form of augmented neuronal activity may be responsible for increasing seizure susceptibility. Although the EEGs are not completely normal in the HGF/*Plaur* mice, abnormal spikes are substantially reduced. These results suggest that postnatal supplementation of HFG/SF is sufficient to reduce seizure susceptibility and severity. Whether there are residual deficits in the ability of PV^+ interneurons to orchestrate gamma oscillations is an open question raised by this work.

In addition to frequent seizures, *Plaur* mice demonstrate increased anxiety in behavioral tests. One test developed by Crawley and colleagues, measures time spent exploring a brightly lit arena or escaping into a darkened box (11). Higher anxiety is associated with spending more time in the safe dark box instead of exploring the arena. Wild-type mice show a slight preference for the dark box, avoiding the brightly lit areas 55% of the time. *Plaur* mice avoid the brightly lit areas more than 70% of the time. Notably, HGF/*Plaur* mice are indistinguishable from wild-type mice in this measure of anxiety. Similar findings were obtained following elevated plus-maze testing.

Taken together, the study by Bae et al., in combination with previous work, indicates that replenishing HGF/SF perinatally not only enables immature PV^+ interneurons to complete their migrations in a *Plaur* mutant background, it also eliminates an anxiety-like phenotype and reduces EEG abnormalities. Because HGF/SF-MET and uPA signaling are important determinants in cell motility, future studies may provide mechanistic explanations for how interneurons migrate into specific cortical layers and areas. As additional experimental tools are developed to systematically manipulate putative guidance molecules for GABAergic interneurons, it can be expected that the unique roles played by different types of interneurons in mood, affect, and memory will be ascertained.

by Janice R. Naegele, PhD

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Responsiveness of Ictaform Discharges to Pharmacotherapy: The Bigger They Are, the Harder They Fall

Antiepileptic Drugs Abolish Ictal But Not Interictal Epileptiform Discharges In Vitro. D'Antuono M, Köhling R, Ricalzone S, Gotman J, Biagini G, Avoli M. *Epilepsia* 2010;51(3):423–431. PURPOSE: We established the effects of the antiepileptic drugs (AEDs) carbamazepine (CBZ), topiramate (TPM), and valproic acid (VPA) on the epileptiform activity induced by 4-aminopyridine (4AP) in the rat entorhinal cortex (EC) in an in vitro brain slice preparation. METHODS: Brain slices were obtained from Sprague-Dawley rats (200–250 g). Field and intracellular recordings were made from the EC during bath application of 4AP (50 μm). AEDs, and in some experiments, picrotoxin were bath applied concomitantly. RESULTS: Prolonged (>3 s), ictal-like epileptiform events were abolished by CBZ (50 μm), TPM (50 μm), and VPA (1 mm), whereas shorter (<3 s) interictal-like discharges continued to occur, even at concentrations up to 4-fold as high. γ-Aminobutyric acid $(GABA)_A$–receptor antagonism changed the 4AP-induced activity into recurrent interictal-like events that were not affected by CBZ or TPM, even at the highest concentrations. To establish whether these findings reflected the temporal features of the epileptiform discharges, we tested CBZ and TPM on 4AP-induced epileptiform activity driven by stimuli delivered at 100-, 10-, and 5-s intervals; these AEDs reduced ictal-like responses to stimuli at 100-s intervals at nearly therapeutic concentrations, but did not influence shorter interictal-like events elicited by stimuli delivered every 10 or 5 s. CONCLUSIONS: We conclude that the AED ability to control epileptiform synchronization in vitro depends mainly on activity-dependent characteristics such as discharge duration. Our data are in keeping with clinical evidence indicating that interictal activity is unaffected by AED levels that are effective to stop seizures.

COMMENTARY

At first glance, the recent paper by D'Antuono and colleagues seems to support the long-held view that antiepileptic drugs (AEDs) are highly effective at suppressing seizures but may be less effective with interictal phenomena. The authors elicited epileptiform activity in the in vitro entorhinal cortex by exposing brain slices to the potassium blocker 4-aminopyridine (4AP), a commonly used experimental convulsant, and tested three AEDs. None of the AEDs tested had a significant effect on interictal burst frequency, even when tested at twice the dose effective for abolishing ictal discharges. From a clinical perspective, does it really matter if AEDs fail to suppress interictal discharges? It has long been assumed that the presence or frequency of interictal spikes on EEG is not necessarily an indication of poor seizure control or AED failure (1), and the dose of AED therapy generally is not increased to try to suppress interictal activity, as these discharges are clinically silent. Instead, the general consensus has been to use AEDs only to suppress clinically expressed seizures. Clinicians are therefore taught to take a good history and treat the patient, not the EEG.

There is growing controversy, however, regarding the significance of interictal activity. Although interictal and ictal discharges are both believed to arise from the epileptic focus, questions have been raised as to whether they are part of one continuum or have independent generation mechanisms. Results from animal studies have not always been in agreement with those from clinical studies. Some experimental data have suggested that interictal activity may actually be protective, suppressing the expression of full-blown ictal events (2,3), but in vivo clinical findings do not seem to support this view (4,5). This area of research was a subject of a point–counterpoint series of articles in the November–December 2006 issue of *Epilepsy Currents* (6–8).

However, any attempt to draw conclusions regarding the significance of interictal activity by reviewing the literature is further muddied by the fact that the clinicians and the basic scientists use the terms "ictal" and "interictal" differently.

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