Neuregulation: NRG1 Tames Interneurons and Epilepsy

Neuregulin 1 Regulates Excitability of Fast-Spiking Neurons Through Kv1.1 and Acts in Epilepsy.

Li KX, Lu YM, Xu ZH, Zhang J, Zhu JM, Zhang JM, Cao SX, Chen XJ, Chen Z, Luo JH, Duan S, Li XM. *Nat Neurosci* 2011;15:267–273.

Dysfunction of fast-spiking, parvalbumin-positive (FS-PV) interneurons is implicated in the pathogenesis of epilepsy. ErbB4, a key Neuregulin 1 (NRG1) receptor, is mainly expressed in this type of interneurons, and recent studies suggest that parvalbumin interneurons are a major target of NRG1-ErbB4 signaling in adult brain. Thus, we hypothesized that downregulation of NRG1-ErbB4 signaling in FS-PV interneurons is involved in epilepsy. We found that NRG1, through its receptor ErbB4, increased the intrinsic excitability of FS-PV interneurons. This effect was mediated by increasing the near-threshold responsiveness and decreasing the voltage threshold for action potentials through Kv1.1, a voltage-gated potassium channel. Furthermore, mice with specific deletion of ErbB4 in parvalbumin interneurons were more susceptible to pentylenetetrazole- and pilocarpine-induced models of epilepsy. Exogenous NRG1 delayed the onset of seizures and decreased their incidence and stage. Moreover, expression of ErbB4, but not ErbB2, was downregulated in human epileptogenic tissue. Together, our findings suggest that NRG1–ErbB4 signaling contributes to human epilepsy through regulating the excitability of FS-PV interneurons. ErbB4 may be a new target for anticonvulsant drugs in epilepsy.

Neuregulin 1 Represses Limbic Epileptogenesis Through ErbB4 in Parvalbumin-Expressing Interneurons.

Tan GH, Liu YY, Hu XL, Yin DM, Mei L, Xiong ZQ. *Nat Neurosci* 2011;15:258–266.

Epilepsy is a common and refractory neurological disorder, but the neuronal regulatory mechanisms of epileptogenesis remain largely unclear. Activity-dependent transcription of genes for neurotrophins such as brain-derived neurotrophic factor (BDNF) has been shown to promote epileptogenesis; however, little is known about factors that may act as intrinsic, homeostatic or counterbalancing mechanisms. Using rodent models, here we show that limbic seizure activity upregulated NRG1-ErbB4 signaling and that epileptogenesis was inhibited by infusing NRG1 intracerebrally but exacerbated by neutralizing endogenous NRG1 with soluble ErbB4 extracellular domain, by inhibiting ErbB4 activation or by deleting the Erbb4 gene. Furthermore, specific depletion of ErbB4 in parvalbumin-expressing interneurons abolished NRG1-mediated inhibition of epileptogenesis and promoted kindling progression, resulting in increased spontaneous seizures and exuberant mossy fiber sprouting. In contrast, depleting ErbB4 in CaMKIIα-positive pyramidal neurons had no effect. Thus, NRG1-induced activation of ErbB4 in parvalbumin-expressing inhibitory interneurons may serve as a critical endogenous negative-feedback mechanism to suppress limbic epileptogenesis.

Commentary

Synaptic inhibition is a complex and highly regulated process, and it plays a critical role in the brain's susceptibility to seizures. All natural modulators of inhibitory circuits are thus immediate suspects in the basic mechanisms of epilepsy and its prevention. Recent studies by Li et al. and Tan et al. reveal a novel function for neuregulin 1 (NRG1) as both an enhancer of inhibition and a repressor of seizures.

NRG1 belongs to the epidermal growth factor (EGF) family of proteins. As trophic factors, some of these proteins guide the development and organization of brain tissue by stimulating axonal outgrowth and guidance, glial cell differentiation, and synapse formation, among other things. NRG1 interacts with ErbB receptor tyrosine kinases and has exceptionally high affinity for ErbB4. Among the neuregulins, NRG1 stands out for the unusually large number of isoforms created by alternative splicing of its gene. The eclectic biological roles of the NRG1-ErbB4 signaling pathway have been extensively reviewed elsewhere (1, 2).

Interest in NRG1-ErbB4 signaling rose enormously when it was implicated as a risk gene in association studies of schizophrenic populations. Compelling connections between malfunctions of this pathway and the pathogenesis of schizophrenia have not yet been identified (3). Intriguingly, though, it is clear in rodents and primates that ErbB4 is expressed

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mainly by specific subpopulations of GABAergic interneurons in the neocortex and hippocampus (4); moreover, NRG1-ErbB4 activation enhances GABAergic transmission (5, 6). Considering the importance of inhibitory circuits for preventing rampant excitation and seizures, it was natural to search for a role of NRG1-ErbB4 in epilepsy.

Both Tan et al. and Li et al. focused on the most common subtype of GABAergic neurons in cortical circuits, those characterized by exceptionally brief action potentials and high maximal firing rates (the "fast spiking," or FS, cells). FS cells are also identified by their expression of the calcium-binding protein parvalbumin (PV). The axons of FS-PV interneurons form GABA-releasing synapses on the soma, axon initial segment, and proximal dendrites of neighboring pyramidal neurons and exert fast, powerful inhibitory effects. ErbB4 is highly expressed in FS-PV interneurons, and they provide the critical link between NRG1-ErbB4 signaling and the excitability of the neuronal network. When Li et al. selectively deleted ErbB4 in FS-PV cells, mice became more susceptible to seizures induced by the convulsants pentylenetetrazole and pilocarpine. Conversely, intraventricular infusion of NRG1 reduced the severity of the seizures but was ineffective when ErbB4 was blocked or deleted. In complementary experiments, Tan et al. showed that impairment of the NRG1-ErbB4 signaling pathway in FS-PV cells—but not in pyramidal cells—accelerated and exacerbated kindling-induced seizures. The same manipulations even altered long-term structural alterations that follow chronic seizures; NRG1 treatment during kindling nearly prevented the sprouting of the excitatory mossy fibers while blocking or deleting ErbB4 enhanced sprouting.

These experiments convincingly link NRG1-ErbB4 signaling, FS-PV interneurons, seizures, and epileptogenesis. But how does NRG1 affect the interneurons and their inhibitory synapses? Li et al. made another major contribution by revealing the mechanism: ErbB4 directly controls the intrinsic excitability of FS-PV cells by regulating a specific potassium channel. They recorded directly from these interneurons in brain slices taken from mice genetically engineered to express green fluorescent protein in FS-PV cells. Direct application of NRG1 enhanced the excitability of the neurons by selectively reducing their spike threshold, and ErbB4 was necessary for this effect. Furthermore, the authors identified the target of ErbB4 as Kv1.1, a voltage-gated potassium channel that is localized to the axon initial segments of cortical interneurons. Kv1.1 activates in the subthreshold voltage range, implying that it reduces interneuron excitability. When endogenous NRG1 effects were blocked, Kv1.1 currents nearly doubled and firing responses of the interneurons were sharply reduced. Finally, using immunoprecipitation, Li et al. showed that activation of the NRG1-ErbB4 pathway increases tyrosine phosphorylation of the channel, a process known to suppress similar potassium currents (7). Because ErbB4 is itself a tyrosine kinase, it is tempting to suggest that this receptor acts directly on Kv1.1 channels situated close by, constituting a quick pathway to modulate membrane excitability.

Altogether, the two sets of authors provide a compelling mechanism by which NRG1-ErbB4 signaling can profoundly and selectively enhance the responsiveness of interneurons, thereby increasing the strength of inhibition in local circuits and repressing seizures and epileptogenesis. Both groups suggest that ErbB4 is an obvious target for anticonvulsant drug development.

More generally, the results of Li et al. highlight the remarkably diverse functions of neurotrophins. It is increasingly evident that these proteins, which traditionally regulate structure and development, also have rapid and relatively direct effects on ion channels and neuronal excitability. The distinction between neurotrophins and neurotransmitters has been gradually blurring (8). Classically, identifying a transmitter requires demonstrating its release by relevant activity, its presence in adequate quantities, and its postsynaptic effects via specific receptors. Li et al. have fulfilled the latter two conditions, demonstrating the presence of endogenous NRG1 in the cortex and its effects when activating its receptor ErbB4. The activity-dependent release of NRG1 remains far less explored. Stability of a neural system requires that any modulator of inhibition must itself be regulated by network activity-dependent feedback. Seizures, an extreme level of activity, stimulate the expression of NRG1, as demonstrated by Tan et al. and others (9). Activity even seems to change NRG1 isoform expression profile (10), enhancing most dramatically the main isoform expressed by astrocytes. Tan et al. also showed that *Nrg1* mRNA increases after a single seizure in kindled rats, peaks after 3 hours and returns to baseline within 24 hours. The release of the soluble form of NRG1 seems to be via proteolytic cleavage during electrical activity, and its release rate indeed depends on the frequency of stimulation (11). Many questions remain, but NRG1 probably deserves the title of neurotransmitter.

Finally, it is evident from both studies that disruption of NRG1-ErbB4 signaling promotes seizures, but how likely is it that defects in this pathway cause any of the human epilepsies? Li et al. found a significant reduction in the expression of ErbB4 (but not ErbB2) in brain tissue from temporal lobe epilepsy patients compared with tissue from nonepileptic controls. This is an intriguing correlation that may indicate a cause of seizures, an effect of chronic epilepsy, or something entirely unrelated. Demonstrating that defects in this signaling pathway cause specific epileptic syndromes will require further genetic and functional studies.

by Yael Amitai, MD, and Barry W. Connors, PhD

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