



IS THE TYROSINE KINASE B RECEPTOR A TARGET FOR PREVENTING EPILEPSY?

Brain-derived Neurotrophic Factor mRNA and Protein Are Targeted to Discrete Dendritic Laminas by Events That Trigger Epileptogenesis

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Dendritic targeting of messenger RNA (mRNA) and local protein synthesis are mechanisms that enable neurons to deliver proteins to specific postsynaptic sites. Here we demonstrate that epileptogenic stimuli induce a dramatic accumulation of brain-derived neurotrophic factor (BDNF) mRNA and protein in the dendrites of hippocampal neurons *in vivo*. BDNF mRNA and protein accumulate in dendrites in all hippocampal subfields after pilocarpine seizures and in selected subfields after other epileptogenic stimuli (kainate and kindling). BDNF accumulates selectively in discrete dendritic laminas, suggesting targeting to synapses that are active during seizures. Dendritic target-

ing of BDNF mRNA occurs when the cellular changes that underlie epilepsy are occurring and is not seen after intense stimuli that are nonepileptogenic, including electroconvulsive seizures and high-frequency stimulation. MK-801, an *N*-methyl-D-aspartate (NMDA) receptor antagonist that can prevent epileptogenesis but not acute seizures, prevents the dendritic accumulation of BDNF mRNA, indicating that dendritic targeting is mediated via NMDA-receptor activation. Together, these results suggest that dendritic accumulation of BDNF mRNA and protein play a critical role in the cellular changes leading to epilepsy.

Exacerbated Status Epilepticus and Acute Cell Loss, but No Changes in Epileptogenesis, in Mice with Increased Brain-derived Neurotrophic Factor Signaling

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Several studies suggest that brain-derived neurotrophic factor (BDNF) can exacerbate seizure development during status epilepticus (SE) and subsequent epileptogenesis in the adult brain. Conversely, evidence exists for the protective effect of BDNF. To study this controversy, we induced SE with kainate in transgenic mice with increased BDNF signaling due to TrkB overexpression. Transgenic mice experienced a more severe SE than wild-type animals did. Furthermore, they had increased acute hippocampal neuronal loss when assessed at 48 hours after SE. The effect of TrkB overexpression on the development of epilepsy, chronic neuronal death, mossy fiber sprouting, and neurogenesis were studied at 4.5 months after kainate-induced

SE. No differences were found in the rate of epileptogenesis, severity of epilepsy, or cellular markers of network reorganization between transgenic and wild-type mice. No differences between genotypes were observed in TUC-4 staining, indicating no effect of TrkB overexpression to immature neuron numbers. Instead, in cresyl violet-stained preparations, the highest density of neurons was found in untreated transgenic mice, suggesting a favorable effect of TrkB overexpression on the survival of neurons in the hippocampus. Our data support the role of BDNF and TrkB signaling in seizure generation and acute cellular damage after SE. Long-term outcome was not, however, exacerbated by TrkB overexpression.

Conditional Deletion of TrkB but Not BDNF Prevents Epileptogenesis in the Kindling Model

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Epileptogenesis is the process whereby a normal brain becomes epileptic. We hypothesized that the neurotrophin brain-derived neurotrophic factor (BDNF) activates its receptor, TrkB, in the hippocampus during epileptogenesis and that BDNF-mediated activation of TrkB is required for epileptogenesis. We tested these hypotheses in *Synapsin-Cre* conditional *BDNF*^{-/-} and *TrkB*^{-/-} mice by using the kindling model. Despite marked reductions of BDNF expression, only a modest impairment of epileptogenesis and increased hippocampal TrkB activation were detected in *BDNF*^{-/-} mice. In con-

trast, reductions of electrophysiologic measures and no behavioral evidence of epileptogenesis were detected in *TrkB*^{-/-} mice. Importantly, *TrkB*^{-/-} mice exhibited behavioral end points of epileptogenesis, tonic-clonic seizures. Whereas TrkB can be activated, and epileptogenesis develops in *BDNF*^{-/-} mice, the plasticity of epileptogenesis is eliminated in *TrkB*^{-/-} mice. Its requirement for epileptogenesis in kindling implicates TrkB and downstream signaling pathways as attractive molecular targets for drugs for preventing epilepsy.

COMMENTARY

If epilepsy is to be prevented, it is important to identify the sequence of events after a neurological insult that leads to recurrent spontaneous seizures. A series of studies identified activation of *N*-methyl-D-aspartate (NMDA) receptors as an early event in the process of epileptogenesis. Since the identification of NMDA-receptor activation as a key switching mechanism that initiates epileptogenesis, the trail appeared to grow cold. Among the candidate mechanisms that could translate NMDA-receptor activation into permanent alterations in neuronal circuits and excitability have been growth factors, including brain-derived growth factor (BDNF). Although much evidence exists that BDNF is involved in the development of epilepsy, it now appears that a primary target of BDNF, the tyrosine kinase B (TrkB) receptor, plays a more important role in epileptogenesis.

BDNF is a secreted trophic factor that, along with neurotrophin-4/5 (NT-4/5) acts as a high-affinity ligand for the TrkB receptor. Neurotrophin-3 (NT-3) binds to the TrkB receptor with low affinity. Characteristic neuropathologic changes of mesial temporal sclerosis, such as neuron loss and axonal sprouting; plastic changes in neuronal networks and synapses; neurogenesis; and dendritic outgrowth (1) can be mediated by BDNF and TrkB activation (2). Neural activity affects BDNF- and TrkB-mediated signaling (3) and promotes effects of BDNF on neuron survival and dendritic arborization. Neuronal depolarization increases levels of BDNF mRNA, whereas neuronal hyperpolarization decreases levels of BDNF mRNA. Furthermore, high-frequency neuronal activity and synaptic transmission elevate TrkB receptors on the surface of cultured hippocampal neurons (4). In turn, increased BDNF induces neuronal

hyperexcitability and promotes long-term potentiation (LTP) of excitatory synaptic transmission (3). Moreover, a low concentration of BDNF, together with brief depolarization of a presynaptic neuron, potentiates neurotransmitter release (5). BDNF- and TrkB-mediated signaling also have been directly linked to epileptogenesis (6). Seizure activity induces a rapid increase in BDNF mRNA expression and TrkB activity, whereas status epilepticus (SE) increases both BDNF mRNA and protein. Exogenous BDNF provokes spontaneous seizures and enhances seizure propagation in animal models. Furthermore, overexpression of BDNF in transgenic mice causes increased seizure severity and epileptiform-evoked responses in the hippocampus.

Recent studies by Lahtinen et al., He et al., and Tongiorgi et al. provide further evidence for the role of BDNF and TrkB-mediated signaling in epileptogenesis by using various models of epilepsy, including kainate, kindling, and pilocarpine. Their results indicate that whereas conditional *BDNF*^{-/-} mice showed almost no differences in epileptogenesis from wild-type controls, significant cellular alterations of BDNF mRNA and protein occurred during the development of epilepsy. These studies also demonstrated that epileptogenesis was inhibited in conditional *TrkB*^{-/-} mice, although TrkB overexpression had little effect on the development of epilepsy.

Lahtinen et al. analyzed the induction of SE in transgenic mice with overexpression of TrkB by using a kainate model of epilepsy and found that it did not affect epileptogenesis. No differences were found in the rate of epilepsy induction or in chronic changes of network organization, sprouting,

neurogenesis, or cellular damage. However, TrkB overexpression did affect SE severity. The transgenic mice had more severe SE than did wild-type mice, reflected by an increased number of seizures in all dose groups and more severe seizures, with longer duration of SE at low-kainate dose, compared with wild-type animals. The transgenic mice also had increased acute hippocampal neuronal loss at 48 hours.

He et al. (2004) evaluated epileptogenesis in conditional BDNF^{-/-} and TrkB^{-/-} mice by using a kindling model of epilepsy. The TrkB^{-/-} mice showed no behavioral evidence of epileptogenesis, although seizures were capable of being induced in a manner similar to those with wild-type controls. Surprisingly, however, the BDNF^{-/-} mice exhibited only a modest impairment of epileptogenesis. Importantly, these studies suggest that TrkB, but not BDNF, is required for epileptogenesis and that other TrkB-receptor ligands, such as NT-4/5 or NT-3, may act as compensatory mechanisms for BDNF during epileptogenesis. Indeed, although BDNF was almost absent from dentate granule and CA3 pyramidal cells in BDNF^{-/-} mice, tyrosine kinase activation was detected in parts of the mossy fiber pathway. When levels of other neurotrophins were evaluated, the hippocampus of BDNF^{-/-} mice showed an increase in NT-3 but not NT-4/5 protein, suggesting NT-3 as the main compensatory mechanism for BDNF in epileptogenesis.

Tongiorgi et al. investigated the subcellular localization of BDNF mRNA and protein during epileptogenesis by using the pilocarpine, kainate, and kindling models of epilepsy. They demonstrated that epileptogenic stimuli induced significant accumulation of BDNF mRNA and protein in distal dendrites of hippocampal neurons *in vivo*. These effects were not seen in nonepileptogenic stimuli, such as electroconvulsive seizures and high-frequency stimuli, and the NMDA antagonist MK-801, known to prevent epileptogenesis but not acute seizures, prevented this dendritic accumulation. BDNF mRNA was targeted to the most highly activated synapses during seizures, synapses that correspond closely to the terminal fields of the mossy fibers or of the commissural–associational (C/A) projection system. Moreover, in chronically spontaneously-seizing, pilocarpine-treated animals, increased dendritic protein localization did not correlate with increased dendritic mRNA levels, suggesting that the localization of BDNF mRNA may allow local synthesis of BDNF protein.

These three studies help elucidate the role of BDNF during epileptogenesis. Tongiorgi et al. suggest that redistribution of BDNF during the development of seizures may be an important step of epileptogenesis. Localization of BDNF to distal dendrites may allow BDNF to potentiate local synapses of the mossy fiber pathway and C/A projection system. However, evidence from He et al. implies that this local BDNF potentiation may not be necessary for epileptogenesis. Their results showed relatively normal epileptogenesis in animals with inhibited

BDNF expression. The study of Lahtinen et al. did not directly test actions of BDNF. The transgenic mice overexpressing TrkB used in their study are not only a model of increased BDNF signaling, as signaling from NT-4/5 and NT-3 as well as the other ligands of TrkB also may be heightened. NT-4/5 and NT-3 may act as compensatory mechanisms for BDNF during epileptogenesis. He et al. demonstrated a significant increase in NT-3 protein in the hippocampus of their BDNF^{-/-} mice.

The three studies also illuminate the function of TrkB during epileptogenesis. He et al. effectively demonstrated that intact TrkB-mediated signaling is critical for epileptogenesis. Although previous experiments from this laboratory showed that blockade of TrkB-mediated signaling, with intraventricular infusion of ligand-scavenging TrkB receptor bodies, only partially reduced epileptogenesis (7), these receptor bodies may have had a limited spatial distribution. In contrast, Lahtinen et al. suggested that overexpression of TrkB had little effect on epileptogenesis. This may occur because TrkB-mediated signaling has an upper limit of activation beyond which further enhancement of TrkB expression produces no effect. Nonetheless, TrkB overexpression did exacerbate acute features of SE. Important differences in outcome also may have occurred because the two studies used different models—He and colleagues used kindling to induce epilepsy, whereas Lahtinen et al. used kainate. A high mortality rate associated with the kainate model likely confounds the measurement of epileptogenesis. It is believed that severity and duration of SE can affect the propensity for the development of epilepsy. However, if animals that undergo severe SE die, any study of subsequent development of epilepsy is likely to be compromised.

Furthermore, these studies emphasize differences between the role of BDNF and TrkB signaling in the process of epileptogenesis. As previously mentioned, BDNF is only one of three ligands of the TrkB receptor, and thus TrkB may be activated by other neurotrophins during the process of epileptogenesis. Immunocytochemical evidence indicates that TrkB is more abundant at synaptic and extrasynaptic glutamatergic and GABAergic receptors than is BDNF (8), suggesting that TrkB is activated by ligands other than BDNF. Therefore, although BDNF may contribute to epileptogenesis *in vivo*, TrkB activation appears to be necessary. The immunocytochemical results also imply that TrkB may monitor numerous additional biologic activities of receptors. Recent evidence suggests that TrkB regulates clustering of glutamatergic and GABAergic receptors (9).

The critical role of TrkB in epileptogenesis suggests that it is a potential target for preventing the development of epilepsy after a neurologic insult. Remaining questions include which of the multiple TrkB-mediated signaling cascades may be responsible for inducing epileptogenesis. The signaling pathway that is activated by the Shc site of the TrkB receptor, which is

implicated in neuronal survival, differentiation, and neurite outgrowth, has previously been shown by He et al. to not contribute to the development of epilepsy (10). Other TrkB-mediated signals, such as protein kinase C, should be investigated as possible mediators of epileptogenesis. Numerous of these TrkB-activated pathways might participate in the sequence of events leading to recurrent spontaneous seizures after a neurologic insult.

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