Commentary

Kinases are ubiquitous enzymes that regulate cellular function by the simple act of transferring a phosphate group to a serine, threonine, or tyrosine residue on a protein substrate. Long a subject of interest at a basic science level, kinases have more recently become the focus of drug development for a number of diseases. Imatinib, an inhibitor of the tyrosine kinase abl, revolutionized the treatment of chronic myelogenous leukemia and became a blockbuster drug. Numerous other new drugs—mostly aimed at oncologic applications—have followed, and drug development against kinases now represents a major focus of pharmaceutical companies (1).

Interest in kinases and phosphorylation signaling is spreading to the epilepsy field as well. The recognition that the kinase mammalian target of rapamycin (mTOR) is hyperactivated in tuberous sclerosis (TS) has stimulated multiple investigations, demonstrating that pharmacologic mTOR inhibition is an effective antiepileptic treatment in humans with TS (2). Several investigators have taken this a step further, testing whether mTOR inhibition might be effective in animal models of acquired epilepsy. Numerous other new drugs—mostly aimed at oncologic applications—have followed, and drug development against kinases now represents a major focus of pharmaceutical companies (1).

While there is no clear evidence that mTOR inhibition may exert an antiepileptic action and possibly even an antiepileptogenic effect—that is, delaying or preventing the development of epilepsy after a neural insult (3, 4).

However, there is no priori reason that mTOR should be the only kinase involved in the development of acquired epilepsy; likely, multiple phosphorylation signaling pathways are activated following a neural insult. The tyrosine kinase TrkB is the latest candidate effector of epileptogenesis. As the current study by Liu et al. demonstrates, TrkB appears to be a critical mediator of epileptogenesis in at least one model of acquired epilepsy. TrkB is the receptor for brain-derived neurotrophic factor (BDNF), a molecule that had been widely hypothesized to mediate epileptogenesis. However, the investigators of the present study had previously demonstrated that genetic deletion of BDNF only modestly impaired development of kindling, while deletion of TrkB virtually abolished kindling (5). This focused attention away from BDNF and towards TrkB as a potential mediator of epileptogenesis in animal models.

It might be argued that kindling is not a sufficiently robust model from which to draw conclusions about epileptogenesis given (in most cases) a lack of spontaneous seizures in kindled animals. The current study takes this issue head-on by using kainic acid (KA) injected into the amygdala to produce chronically epileptic mice. The authors also employed a clever technique to circumvent the lack of selective TrkB pharmacologic inhibitors: a transgenic mouse engineered with a TrkB mutation that renders its TrkB receptors susceptible to inhibition by a novel small molecule (1NMPP1). This enabled the investigators to selectively block TrkB in the mutant mice, while wild-type mice were unaffected by the novel inhibitor.

With this tool in hand, the investigators used a straightforward and rigorous experimental design to test whether TrkB inhibition affected epileptogenesis following KA injection. They implanted a single depth EEG electrode in the hippocampus to monitor seizure occurrence and then injected KA in the contralateral amygdala. After 40 min of status epilepticus (SE), they administered benzodiazepines and started TrkB inhibitor treatment. The inhibitor was continued for two weeks, during which EEG was continuously monitored. Three weeks after the inhibitor was stopped, a repeat week of EEG monitoring was used to evaluate whether animals remained seizure-free.

Transient Inhibition of TrkB Kinase after Status Epilepticus Prevents Development of Temporal Lobe Epilepsy.


Temporal lobe epilepsy is the most common and often devastating form of human epilepsy. The molecular mechanism underlying the development of temporal lobe epilepsy remains largely unknown. Emerging evidence suggests that activation of the BDNF receptor TrkB promotes epileptogenesis caused by status epilepticus. We investigated a mouse model in which a brief episode of status epilepticus results in chronic recurrent seizures, anxiety-like behavior, and destruction of hippocampal neurons. We used a chemical-genetic approach to selectively inhibit activation of TrkB. We demonstrate that inhibition of TrkB commencing after status epilepticus and continued for 2 weeks prevents recurrent seizures, ameliorates anxiety-like behavior, and limits loss of hippocampal neurons when tested weeks to months later. That transient inhibition commencing after status epilepticus can prevent these long-lasting devastating consequences establishes TrkB signaling as an attractive target for developing preventive treatments of epilepsy in humans.
Biochemical analysis of TrkB activity showed that TrkB was hyperactivated within hours following SE, remained so for at least several days, and was effectively reduced to control levels by the novel inhibitor.

TrkB inhibition caused an impressive reduction in the development of epilepsy. Out of 10 treated mutant mice, only two showed seizures in the first two weeks post-SE (during inhibitor treatment), while only one remained epileptic in weeks 5–6 post-SE after the inhibitor had been stopped. In comparison, 100% of wild-type animals treated with inhibitor (which was ineffective in blocking TrkB activity since the mice lack the sensitizing mutation) became epileptic and showed a far higher rate of spontaneous seizures. The investigators went on to show that the treated, non-epileptic mice showed relative preservation of hippocampal pyramidal neuron counts and lacked anxiety-like behaviors that epileptic mice exhibited. Thus, TrkB inhibition robustly protected against the development of epilepsy and some of its behavioral sequelae.

The magnitude of the effect of TrkB inhibition in this study was substantial and the experiments were performed meticulously. Is the case closed that TrkB mediates epileptogenesis? A few issues remain to be explored. In this protocol, the inhibitor was delivered while SE was still ongoing, raising the possibility that its administration somehow attenuated the intensity of the SE insult. To their credit, the investigators quantified EEG power and behavioral seizure scores during SE to dispel concerns that treated animals were not subjected to a similarly intense insult as the controls. Nonetheless, it is hard to know whether some aspect of SE not captured by EEG was affected by treatment. Likewise, inhibitor treatment was continued for two weeks, a time period when untreated animals began to have spontaneous seizures; if the inhibitor has intrinsic antiepileptic properties, it is possible that suppression of early seizures may only delay the onset of epilepsy and not prevent it altogether, as has been shown in a genetic model of epilepsy (6). Ideally, these issues could be addressed by changing the treatment time window so not to overlap SE or the typical onset of spontaneous seizures. A trial of the inhibitor in animals with established epilepsy would also determine whether the drug has intrinsic antiepileptic properties; since post-SE animal models depend on seizures to generate a brain insult, an intervention with antiepileptic efficacy presents a potential confound in determining its antiepilptogenic influence.

Those caveats aside, this study represents a compelling validation of TrkB as a phosphorylation signaling pathway with an important role in epileptogenesis. The identification of downstream effectors of TrkB, as well as upstream activators, will be vital topics of future investigation. If pharmacological development against TrkB signaling proceeds as is occurring with other disease-implicated kinases, the therapeutic potential of TrkB inhibition after neural insult could be explored in a variety of animal models, and, after further validation, perhaps in humans as well.

by Nicholas P. Poolos, MD, PhD

References
Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

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2. First Name Nicholas  Last Name Poolos  Degree MD, PhD

3. Are you the Main Assigned Author? ☑ Yes  ☐ No

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4. Manuscript/Article Title: Stopping epileptogenesis dead in its Trks

5. Journal Issue you are submitting for:

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